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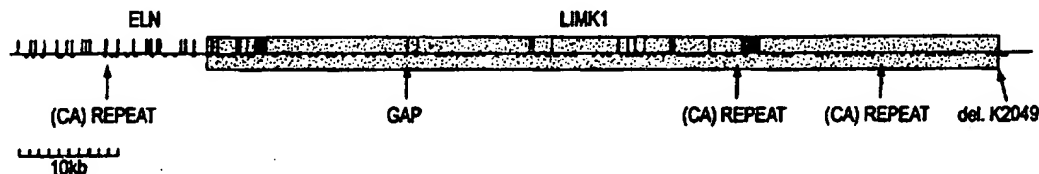
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(54) Title: DIAGNOSIS OF WILLIAMS SYNDROME AND WILLIAMS SYNDROME COGNITIVE PROFILE BY ANALYSIS OF THE PRESENCE OR ABSENCE OF A LIM-KINASE GENE



## (57) Abstract

Williams syndrome (WS) is a developmental disorder that includes poor visuospatial constructive cognition. This syndrome has been studied to identify genes important for human cognitive development. Two families are described which have a partial WS phenotype; affected members have the specific WS cognitive profile and vascular disease, but lack other WS features. Submicroscopic chromosome 7q11.23 deletions cosegregate with this phenotype in both families. DNA sequence analyses of the region affected by the smallest (63.6 kb) deletion revealed two genes, *elastin* (*ELN*) and *LIM-kinase 1* (*LIMK1*). The latter encodes a novel protein kinase with LIM domains and is strongly expressed in the brain. Because *ELN* mutations cause vascular disease but not cognitive abnormalities, these data implicate *LIMK1* hemizyosity in impaired visuospatial constructive cognition.

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TITLE OF THE INVENTION

## DIAGNOSIS OF WILLIAMS SYNDROME AND WILLIAMS SYNDROME COGNITIVE PROFILE BY ANALYSIS OF THE PRESENCE OR ABSENCE OF A LIM-KINASE GENE

5           This application was made with Government support under Grant No. R01HL4807 from the NHLBI, Grant No. R01HD29957 from the NICHD, and Grant No. M01-RR00064 from the Public Health Service. The United States Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

10           The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated by reference, and for convenience are referenced in the following text by author and are listed alphabetically by author in the appended bibliography.

15           The ability to visualize an object (or picture) as a set of parts and construct a replica of the object from those parts is known as visuospatial constructive cognition. Neuroanatomical studies in humans and animals suggest that neurons in the posterior parietal cortex are critical for this process (Capruso et al., 1995). This cognitive function is likely mediated by a network of neurons capable of parallel processing. The molecular mechanisms underlying development of these networks, however, are not understood.

20           Williams syndrome (WS) is a complex developmental disorder that includes a specific cognitive profile (WSCP) characterized by relative strength in language and auditory rote memory and pronounced weakness in visuospatial constructive cognition (Udwin et al., 1987; Morris et al., 1988; Dilts et al., 1990; Bellugi et al., 1994; Mervis and Bertrand, in press; Mervis et al., in press). Additional features of WS include congenital heart and vascular disease,  
25           dysmorphic facial features, infantile hypercalcemia, mental retardation, and a characteristic personality. Most individuals with WS have mild or moderate mental retardation (mean IQ ranging from 55-60), but some have borderline normal intelligence or severe mental retardation. The characteristic personality includes excessive friendliness, loquaciousness, oversensitivity to the feelings of others, and extreme anxiety to please. This combination of features results in a  
30           remarkable phenotype that is readily distinguished from other disorders involving mental retardation. The incidence of WS is estimated to be 1 in 20,000 live births.

          The visuospatial constructive cognitive deficit observed in WS is best demonstrated by tasks involving pattern construction. Performance of these tasks depends on an individual's ability to see an object in terms of a set of parts specified by the researcher and then use those

parts to construct a replica of the pictured object. Specifically, individuals are shown a picture of a block design and must construct the corresponding pattern using cubes of varying colors and designs. Individuals with WS typically have difficulty constructing even simple patterns, such as a checkerboard consisting of four cubes. As a result, individuals with WS have marked  
5 difficulty in tasks involving the use of a pattern to assemble an object (e.g. building a model or assembling a simple piece of furniture).

Approximately 77% of individuals with WS have clinically apparent supralvalvular aortic stenosis (SVAS), an obstructive vascular disease (Lowery et al., 1995). SVAS can be inherited as part of WS or as an isolated, autosomal dominant trait (Curran et al., 1993; Ewart et al.,  
10 1993b; Morris et al., 1993; Ewart et al., 1994). SVAS may be associated with some connective tissue abnormalities seen in WS, but other WS features are not observed. In particular, autosomal dominant SVAS is not associated with impaired visuospatial constructive cognition. Recently, genetic linkage and mutational analyses were used to show that mutations in *elastin* (*ELN*) cause autosomal dominant SVAS (Ewart et al., 1993a; Curran et al., 1993; Morris et al.,  
15 1993; Ewart et al., 1994). Known SVAS-associated mutations in *ELN* include a translocation, an intragenic deletion, and missense and nonsense mutations (Curran et al., 1993; Olson et al., 1995; unpublished data).

Because there is a phenotypic link between SVAS and WS, it was hypothesized that mutations involving *ELN* might also contribute to WS. It was discovered that WS results from  
20 submicroscopic deletions of chromosome 7q11.23 (Ewart et al., 1993a). Inherited or *de novo* deletion of one *ELN* allele was identified in 239 of 240 WS individuals (Ewart et al., 1993a; Lowery et al., 1995; and our unpublished data). These data indicated that *ELN* mutations cause isolated, autosomal dominant SVAS and that hemizyosity at the *ELN* locus is responsible for vascular pathology in WS. *ELN* hemizyosity may also account for some connective tissue  
25 abnormalities observed in individuals with autosomal dominant SVAS or WS, including premature aging of skin, some WS facial features, diverticulosis of the bladder and colon, hoarse voice, hernias and joint abnormalities. *ELN* mutations, however, do not account for all features of WS and are not the cause of impaired visuospatial constructive cognition. Because genomic deletions responsible for WS extend well beyond the *ELN* locus (unpublished data), it was  
30 hypothesized that WS is a contiguous gene deletion syndrome (Ewart et al., 1993a).

Here is reported the identification and characterization of two families with a partial WS phenotype, consisting of SVAS, some WS facial features, and impaired visuospatial constructive cognition, but lacking other features of this disorder. Affected members of these families harbor smaller chromosomal deletions (83.6 and ~300 kb) than those identified in individuals with classic WS (>500 kb), an observation that supports the hypothesis that WS is a contiguous gene deletion syndrome (Ewart et al., 1993a; Gilbert-Dussardier et al., 1995). DNA sequence analyses of the 83.6 kb deletion region have revealed, in addition to *ELN*, *LIM-kinase1* (*LIMK1*), a gene which encodes a protein kinase with LIM domains (Mizuno et al., 1994; Bernard et al., 1994). No other genes were identified in the region. Northern and *in situ* hybridization analyses indicate that *LIMK1* is strongly expressed in discrete regions of the brain. Because *ELN* mutations cause vascular disease but not cognitive abnormalities, these data indicate that *LIMK1* hemizygosity contributes to impaired visuospatial constructive cognition in WS.

#### SUMMARY OF THE INVENTION

To identify genes important for human cognitive development, Williams syndrome (WS), a developmental disorder that includes poor visuospatial constructive cognition, has been studied. Two families are here described with a partial WS phenotype; affected members have the specific WS cognitive profile and vascular disease, but lack other WS features. Submicroscopic chromosome 7q11.23 deletions cosegregate with this phenotype in both families. DNA sequence analyses of the region affected by the smallest (83.6 kb) deletion revealed two genes, *elastin* (*ELN*) and *LIM-kinase1* (*LIMK1*). The latter encodes a novel protein kinase with LIM domains and is strongly expressed in the brain. Because *ELN* mutations cause vascular disease but not cognitive abnormalities, these data implicate *LIMK1* hemizygosity in impaired visuospatial constructive cognition.

#### BRIEF DESCRIPTION OF THE FIGURES

Figures 1A and 1B. Co-inheritance of a partial WS phenotype and deletions involving *ELN* and *LIMK1* in kindreds 1895 and 2049. A) Pedigree structure and phenotypic assignments for K1895 are shown. Individuals with SVAS are indicated by filled, upper half-circles (females) or squares (males). Individuals with the WSCP are indicated by filled, lower half-circles or squares. Phenotypically unaffected individuals are indicated by empty circles or

squares. Individuals I-2, II-2, and II-4 were phenotypically affected with both SVAS and the WSCP. No features of WS were identified in other members of this kindred. Individuals harboring an ~300 kb deletion of chromosome 7q11.23, including the entire *ELN* and *LIMK1* genes, are indicated by a D. Note that this deletion cosegregates with the SVAS/WSCP phenotype in this family. B) Phenotypic designations for members of K2049 are as described for Figure 1A, except that an uncertain phenotype is indicated by stippling. Oligonucleotide primers 403f (5'-CCTACCTTTCCTGCTGCAAT-3' SEQ ID NO:37) and 403r (5'-AAAAAGAGGCCGGGTATGGT-3' SEQ ID NO:38) were used to define a novel 403-bp PCR product that spans the 83.6-kb deletion in affected members of this family. The results of PCR analyses are shown below in the lane corresponding to each symbol. Note that this 83.6-kb deletion cosegregates with SVAS/WSCP in this family but that penetrance is incomplete.

Figure 2. Physical map of the deletions identified in K1895 and K2049. Idiogram of chromosome 7 and a contiguous set of cosmids and phage  $\lambda$  from chromosome 7q11.23 are shown. The relative locations and the structures of *ELN* and *LIMK1* are indicated; exons are indicated by vertical bars extending above the horizontal lines; repetitive elements (e.g., Alu repeats) are denoted by vertical bars extending below the lower horizontal line; the locations of three d(CA)-repeats are indicated (the *ELN* d(CA)-repeat has been previously defined; Foster et al., 1993). The small 250 bp gap in the sequence contig is immediately 5' of *LIMK1*. *LIMK1* is located 15.4 kb 3' of *ELN* and is in the same orientation. The locations of the ~300 kb deletion identified in K1895 and the 83.6 kb deletion identified in K2049 are indicated by shaded boxes. Note that both deletions disrupt *ELN* and delete *LIMK1*.

Figures 3A and 3B. Predicted structure of LIMK1. A) DNA sequence analyses were used to predict the amino acid sequence of LIMK1. Two possible start sites are indicated by asterisks. The second start site shows slightly better conformity to the Kozak consensus sequence (Kozak, 1989). Individual amino acids involved in zinc-finger formation as part of two LIM domains are indicated by lightly shaded boxes. A DHR domain between residues 165 and 258 is denoted by a darkly shaded box. A possible PEST domain identified in residues 264-289 is indicated by a lightly shaded box. A basic domain located in residues 499-506 (empty box) may mediate nuclear localization. The kinase domain, indicated by horizontal black bars, is divided into eleven subdomains (I-XI). Conserved amino acids in the kinase domain are



indicated by empty boxes (Hanks et al., 1988). B) Schematic representation of *LIMK1* indicating major domains.

Figures 4A-4E. FISH analyses demonstrate hemizyosity of *LIMK1* in individuals with a partial WS phenotype. Labeled *LIMK1* cosmids c138-13c and c1-4a2 were hybridized with metaphase chromosomes from an affected member of K1895 (A) and of K2049 (B), an individual with classic WS (C), an individual with SVAS with a translocation disrupting *ELN* in exon 28 (D), and an individual with SVAS and no chromosomal anomaly (E). Centromere-specific markers are indicated by arrows (chromosome 7 for all individuals and chromosomes 6 and 7 for the translocation). Affected members of K1895, K2049, and classic WS individuals showed *LIMK1* hemizyosity. The individual with SVAS and a t(6p21;7q11) translocation showed hybridization signals on the normal homologue, as well as on the 7q:6q translocation chromosome. An individual with SVAS, with no chromosomal abnormalities, showed *LIMK1* hybridization signals on both chromosome 7 homologues. All individuals showed two hybridization signals for chromosome 7 centromere-specific marker.

Figures 5A-5B. *LIMK1* is expressed strongly in the brain. Figure 5A shows the results of Northern analyses. Human adult, fetal, and brain Northern blots (poly[A]<sup>+</sup> RNA, 2 µg per lane) were hybridized with *LIMK1*, *ELN*, and  $\beta$ -actin probes. *LIMK1* hybridized with an ~3.3 kb mRNA in most tissues examined, with highest expression in both fetal and adult brain. *ELN* also hybridized with an ~3.3 kb mRNA with highest expression in heart, pancreas, and fetal lung. Figure 5B shows a graphic representation of *LIMK1* expression levels after normalization to  $\beta$ -actin.

Figures 6A-6H. *In situ* hybridization analysis of *LIMK1* expression in the nervous system of a Carnegie stage 20 (50 day postovulatory) human embryo. A 625-bp *LIMK1* cRNA probe was labeled with DIG-UTP and visualized using anti-DIG alkaline phosphatase antibody.

(A) Transverse section through rhombencephalon/medulla, fourth ventricle. *LIMK1* expression is seen in the ependymal layer of the fourth ventricle and a lower level of expression extends into the mantle layer. The arrow indicates expression in the medial accessory olivary nucleus on either side of the midline; this area is shown in greater detail in C. (B) Similar section to (A) hybridized with the sense-strand cRNA probe as a negative control. (C) Medial accessory olivary nuclei shown in the center of (A). (D) Transverse section through the cerebellum (c) showing a high level of ependymal expression in the corpus cerebelli (fourth ventricle on the

right and ectoderm on the left). Some expression is visible in the mesenchyme adjacent to the ectoderm, in particular in the presumptive dentate nucleus (arrow). (E) Transverse section through the cervical spinal cord showing generalized expression in the dorsal (top) part of the spinal cord and single-cell staining more ventrally (right). There is also expression in the dorsal root ganglia (d). (F) Section through the wall of the mesencephalon (the ventricle is on the far right); the ependymal layer is on the right and heavily stained, and the mantle layer in the center-left shows many cells expressing *LIMK1*. An arrow indicates the sulcus limitans. (G) Higher magnification of (E), showing the mid-area of the spinal cord, demonstrates a low level of confluent expression in the ependymal layer (right), widespread single-cell staining in the mantle layer (center), and lack of expression in the marginal layer (left). (H) Transverse section through the fifth nerve ganglion shows high expression in the center, in part of the inner ear (lower right, below the scale bar), and in the ectoderm (left). The scale bar represents either 100 $\mu$ m (C, F, and G) or 250  $\mu$ m (A, B, D, E, and H).

## 15 DETAILED DESCRIPTION OF THE INVENTION

Williams syndrome is a contiguous gene disorder resulting from mutations in or deletion of at least three distinct genes. These genes are located on chromosome 7 in the 7q11.23 region. Two of the genes involved in Williams syndrome are *elastin* (*ELN*) and *LIM-Kinase1* (*LIMK1*). A minimum of at least one more gene located greater than 300 kb 3' of *LIMK1* is also involved in Williams syndrome. The identity of this gene or genes has not yet been established. Williams syndrome results from loss of all of the involved genes. Loss of only one or two of the involved genes causes other disorders which involve only some of the aspects of Williams syndrome. A partial loss of functional elastin due to the presence of only one wild-type elastin gene results in the condition known as supravalvular aortic stenosis (SVAS) which is an obstructive vascular disease. Elastin is a structural protein important in large arteries, lungs and skin. A partial loss of both functional elastin and *LIMK1* due to the presence of only one wild-type copy of each of the corresponding genes results in the condition known as Williams syndrome cognitive profile (WSCP). *LIMK1* is a protein kinase which is highly expressed in the brain and is important in visuospatial constructive cognition. A functional loss of not only elastin and *LIMK1* but also at least one more protein encoded by a gene 3' of *LIMK1* results in development of classic Williams syndrome. Persons with SVAS and WSCP have only a subset

of the characteristics seen in persons with classic Williams syndrome. Persons with Williams syndrome have been found to have a deletion of greater than 500 kb in the 7q11.23 region of one chromosome, this deletion including at least a portion of *ELN*, *LIMK1* and at least 300 kb 3' of *LIMK1*. Although some families have been found which show deletions of *ELN* and *LIMK1* but which deletions do not extend far enough 3' to delete a third gene (these families thus being characterized as having WSCP), in 99% of the cases studied a person who has a deletion in *ELN* is found to have a deletion of greater than 500 kb such that the deletion includes not only *ELN* but also *LIMK1* and at least one other gene 3' of *LIMK1* thus resulting in classic Williams syndrome. This is significant in that a hemizygous deletion of *ELN* indicates a 99% chance that the patient has classic Williams syndrome and not simply SVAS or WSCP.

It is here concluded that *LIMK1* hemizygosity contributes to impaired visuospatial constructive cognition in WS. This conclusion is supported by the following observations: 1) SVAS and the WSCP are co-inherited in K1895 and K2049, as well as in classic WS, suggesting that the genes responsible for these two phenotypes are closely linked; 2) *ELN* and *LIMK1* are contiguous genes that are both disrupted by an 83.6-kb deletion that cosegregates with SVAS and the WSCP in K2049; 3) DNA sequence analyses of the 83.6-kb deletion region and 24 kb of flanking sequence revealed only *ELN* and *LIMK1*; no other genes were identified in these sequences; 4) *LIMK1* is highly expressed in the brain, consistent with its possible function in cognitive development; and 5) intragenic deletions and point mutations affecting only *ELN* cause SVAS but no cognitive impairment, indicating that *ELN* hemizygosity is not sufficient to cause impaired visuospatial constructive cognition in WS.

It is also very unlikely that *ELN* mutations are necessary for impaired visuospatial constructive cognition in WS. First, no correlation exists between the severity of the vascular disease and the severity of cognitive impairment in WS. Second, *ELN* is a structural protein that is important for the development of elastic fibers in large arteries, lungs, and skin, but these elastic fibers are not found in the brain. Finally, *ELN* is not expressed in neurons and glial cells of the brain (R. Mecham, personal communication). Therefore, it is concluded that *ELN* mutations and secondary vascular disease are not sufficient, and almost certainly not necessary, for impaired visuospatial constructive cognition in WS.

The argument that *LIMK1* hemizygosity contributes to impaired cognition would be confirmed by the identification of individuals with intragenic mutations of this gene.

Preliminary experiments aimed at ascertainment of such individuals have not been successful. This is not surprising, because these individuals are probably rare and likely have a very subtle phenotype. To exclude the involvement of additional genes in development of the WSCP, the 83.6-kb deletion region and 24 kb of flanking sequence were sequenced. Programs designed to  
5 identify coding regions revealed only two genes, *LIMK1* and *ELN*. While these analyses did not absolutely exclude the presence of a third gene, the sensitivity of the search algorithms was demonstrated by their identification of 15 of the 16 *LIMK1* exons. It is highly likely, therefore, that all genes in this region were detected.

Previous studies of *LIMK1* expression are consistent with a role for this gene in cognitive  
10 development. Northern analyses in rat showed *LIMK1* expression in multiple tissues, with mRNA levels being highest in the brain (Mizuno et al., 1994). Bernard et al. (1994) identified ubiquitous murine embryonal expression, but found significant mRNA levels only in the adult brain. *In situ* hybridization and immunohistochemical studies performed in mice and humans localized *LIMK1* mRNA and protein exclusively to neurons (basal ganglia, Purkinje cells, and  
15 pyramidal neurons; Bernard et al., 1994). Using Northern blot analysis, Pröschel et al. (1995) demonstrated expression of *LIMK1* in adult murine spinal cord, cortex, cerebellum, and placenta, with lower levels of mRNA in several other tissues. *In situ* hybridization of tissues collected during various stages of murine development indicated expression of *LIMK1* in the developing brain, including the subpial layers of the frontal cortex, the midbrain roof, tectum, cerebellum,  
20 and neural epithelium of the olfactory bulb. In the adult mouse, *LIMK1* expression persisted in the cerebral cortex. Our Northern data indicate expression of *LIMK1* in multiple human fetal and adult tissues but mRNA levels were highest in brain. *In situ* hybridization data presented here also indicate that in developing human tissues, *LIMK1* mRNA is predominantly found in brain, a localization consistent with the pattern of *LIMK1* expression in the mouse and rat  
25 (Bernard et al., 1994; Cheng and Robertson, 1995; Nunoue et al., 1995; Pröschel et al., 1995). The discrete organization of *LIMK1* expression in the developing and adult nervous system, with consistent expression in the ependymal layer from which neurons are generated, is consistent with the hypothesis that this gene plays an important role in neural development.

The data suggest that impaired visuospatial constructive cognition in WS results from a  
30 quantitative reduction in *LIMK1* mRNA and protein. This hypothesis is consistent with recent data examining the role of protein kinases in murine development. Impaired long-term

potentiation, spatial learning, and hippocampal development were identified in mice deficient in the brain-specific protein kinases *fyn* (Grant et al., 1992) and the  $\gamma$  isoform of *protein kinase C* (Abeliovich et al., 1993a; Abeliovich et al., 1993b). Although the spatial learning deficits observed in these mice were not directly analogous to impaired visuospatial constructive cognition in humans with WS, the data do indicate a role for kinases in neuronal development.

The function of LIMK1 is not known, but the presence of specific functional domains suggests possibilities. LIM domains are zinc-binding motifs first identified in the developmentally important genes *lin-11*, *isl-1*, and *mec-3* (Freyd et al., 1990; Karlsson et al., 1990; Way and Chalfie, 1988). LIM domains have been identified in isolation, or in combination with homeodomains, and are thought to modulate cell fate and differentiation (Schmeichel and Beckerle, 1994). LIMK1, by contrast, is unique because it contains a kinase domain in addition to two LIM domains. Predicted amino acid sequence analyses also indicate the presence of a possible PEST domain, a type of sequence that is often found in proteins with short half-lives. This observation suggests that levels of LIMK1 may be tightly regulated. Finally, the predicted amino acid sequence of LIMK1 indicates that cytoskeleton and nuclear localization signals may be present. Biochemical and developmental studies of LIMK1 function will be instrumental in defining the role of this protein in human cognitive development.

The phenotypic variability observed in this study results from variable expression and incomplete penetrance, consistent with results of previous studies of autosomal dominant SVAS and WS (Morris et al., 1988; Ewart et al., 1993a). Variable expression of dysmorphic facial features in individuals with isolated SVAS and classic WS have led to diagnostic confusion in the past (Grimm and Wesselhoeft, 1980), but in this and previous studies, it has been shown that individuals with autosomal dominant SVAS have 6 or fewer of the 16 facial features associated with classic WS. These data indicate that *ELN* mutations account for SVAS and some WS facial features, but that hemizygosity of another, contiguous gene accounts for other WS facial features. Continued deletional analyses should help define genes that contribute to the full WS phenotype, including those involved in the facial features, mental retardation, and the WS personality.

The DNA sequence analyses for the present studies revealed a high density of Alu repeats within the region deleted in K2049 (6-fold higher than the estimated mean density throughout the human genome; Hwu et al., 1986; Slightom et al., 1994), a density comparable to

that found in the genomic region associated with DiGeorge syndrome (Budarf et al., 1995). Both WS and DiGeorge syndrome result from chromosomal rearrangements, which might be driven by the highly repetitive nature of the DNA. In this regard, it is interesting to note that we identified Alu sequences at both breakpoints in K2049, suggesting that a recombinational event  
5 between these elements may have been responsible for this deletion. Alu repeats have previously been implicated in an SVAS-associated translocation and in an intragenic deletion of *ELN* (Curran et al., 1993; Olson et al., 1995).

In summary, it has been here discovered that hemizygosity of *LIM-kinase1*, a protein kinase gene expressed in the brain, likely leads to impaired visuospatial constructive cognition in  
10 Williams syndrome. Further elucidation of the physiologic significance of this gene may result from gene targeting experiments in mice. Analyses of LIMK1 function should provide further insight into human cognitive development.

### EXAMPLE 1

#### 15 Identification of individuals with a partial WS phenotype

If WS is a contiguous gene deletion disorder, individuals with a partial WS phenotype should exist. To test this hypothesis, individuals with SVAS were phenotypically characterized for the presence of additional WS features, including facial appearance, the WSCP, the WS personality, and mental retardation. Phenotypic studies included personal interview, physical  
20 examination, two-dimensional and Doppler echocardiography, IQ determination, WS personality assessment, and WSCP analyses.

#### Clinical characterization of participants

Medical records were reviewed and participants were examined by a clinical geneticist. Craniofacial features scored included dolichocephaly, broad brow, periorbital fullness, stellate  
25 iris, bitemporal narrowing, low nasal root, flat mala, full cheeks, long philtrum, small jaw, malocclusion, full nasal tip, wide mouth, full lips, prominent ear lobes, and facial asymmetry. Individuals with classic WS had 9 or more of the 16 features and met the diagnostic criteria of Preus (1984). Affected members of K1895 and K2049 had 0-6 of the 16 facial features and none of these individuals fit the diagnostic criteria for WS. The presence and extent of SVAS was  
30 determined by two-dimensional echocardiography and Doppler blood-flow analyses as described by Ensing et al. (1989). Individuals were scored as affected if there was narrowing of the

ascending aorta demonstrated on echocardiography or if Doppler peak flow velocities were above normal (normal values for adults: aortic 1.0-1.7 m/s, pulmonary 0.6-0.9 m/s; children: aortic 1.2-1.8 m/s, pulmonary 0.7-1.1 m/s). Velocities within 0.2 m/s greater than the normal range were considered weakly positive. Individuals were also scored as positive if SVAS was documented by medical records of cardiac catheterization or surgery.

#### Determination of Williams Syndrome Cognitive Profile

The general pattern of cognitive strengths and weaknesses observed in WS (WSCP) has been described in several laboratories (Udwin et al., 1987; Bellugi et al., 1994; Mervis and Bertrand, in press; Mervis et al., in press), but until now, no formal method for assessment has been available. The profile assessment that was proposed is based on performance on the DAS (Elliot, 1990), a standardized measure of cognitive abilities. The DAS was specifically designed to identify relative strengths and weaknesses in cognitive abilities. The six core subtests assess language, spatial (visuospatial constructive cognition), and reasoning abilities. A diagnostic subtest measures auditory rote memory. Thus, the DAS covers all of the skills included in the cognitive profile associated with WS.

Individuals who met one or more of the following criteria were excluded from having the WSCP:

- i. pattern construction standard score  $\geq$  mean of the core subtest scores (visuospatial constructive ability too high relative to overall level of cognitive abilities)
- ii. pattern construction standard score  $\geq$  digit recall standard score (visuospatial constructive ability too high relative to auditory rote memory ability)
- iii. pattern construction standard score  $\geq$  20th percentile (absolute level of visuospatial constructive ability too high)
- iv. none of the seven subtest standard scores  $>$  1st percentile (absolute level of ability too low).

Individuals who were not excluded were considered to have the WSCP and were evaluated further to determine the strength of their match to the WSCP. A maximum of 4 points could be earned (4 points = excellent fit, 3 points = very good fit, 2 points = good fit, and 0-1 point = poor fit to the WSCP).

- i. digit recall standard score  $>$  mean of the core subtest standard scores (2 points).

- ii. verbal standard scores > pattern construction standard score
  - a. definition standard score (naming vocabulary was used for younger children) > pattern construction standard score (1 point).
  - b. similarities standard score > pattern construction standard score (1 point).

5       The DAS was used for individuals who were at least 2 1/2 years old. For younger children, the WSCP was assessed using the mental scale of the Bayley Scales of Infant Development (Bayley, 1969; Bayley, 1993). The child was considered to have the WSCP if he or she passed a greater proportion of language items attempted than non-language items. Use of the Bayley to determine if a child's cognitive profile is consistent with the WSCP has been  
10       validated in a study comparing very young children with WS to very young children with Down syndrome (Mervis & Bertrand, in press). In the present study, the Bayley measure was used for one child (K1895 II-4), who was 15 months old at the time of assessment.

      Individuals who did not complete the DAS were phenotypically characterized with the Wechsler Adult Intelligence Scale-Revised (WAIS-R) whenever possible. Exclusion criteria for  
15       the WAIS-R are listed below:

- i. block design standard score > digit span standard score
- ii. block design standard score > 20th percentile
- iii. none of the subtest standard scores > 1st percentile

      Individuals who were not excluded on the basis of these criteria were considered to have  
20       a cognitive profile consistent with the WSCP if both their digit recall and similarities standard scores were greater than their block-design standard score. Those individuals who could not complete the entire WAIS-R were given the verbal portion of the WAIS-R and the Developmental Test of Visual-Motor Integration (VMI; Beery, 1989). Individuals were excluded from further consideration for the WSCP if their VMI age equivalent was greater than  
25       10 years. Individuals who were not excluded were considered to have a cognitive profile consistent with the WSCP if their standard score on the verbal portion of the WAIS-R was greater than their standard score on the VMI.

#### Determination of the Williams Syndrome Personality

      Each member of K1895 and K2049 and 9 of the 11 individuals with isolated SVAS were  
30       independently assessed for the WS personality by two or three examiners (inter-rater agreement = 100%). Of the 85 individuals with classic WS, 65 were assessed by two examiners (inter-rater



agreement = 98%) and the remainder by one examiner. Twenty-two of the 65 individuals in the control group were assessed by two examiners (inter-rater agreement = 95%) and the remainder by one examiner. The following seven WS personality characteristics were evaluated: 1) the presence of an appealing personality; 2) excessive friendliness; 3) loquaciousness; 4) extreme sensitivity to others' feelings; 5) excessive anxiousness to please; 6) very high anxiety; and 7) an extreme interest in people. Phenotypic status was based on the number of characteristics that each individual possessed. Individuals with 4 to 7 of the characteristics were classified as having the WS personality; those with 3 were classified as uncertain; and those with 0 to 2 were classified as not having the WS personality.

10 Determination of Mental Retardation/Developmental Delay

Intelligence was assessed using the Bayley for children < 2 1/2 years old, the DAS was used for individuals between the ages of 2 1/2 and 18 years, and the WAIS-R was used for individuals who were 18 years or older. All measures were administered according to standard procedures. Individuals who were at least 6 years old were considered to have mental retardation if their standard score was <70 (>2 standard deviations below the standardization sample mean). Individuals who were less than 6 years old were considered to have developmental delay if their standard score was <70.

Results

Phenotypic assignment with respect to WSCP was based, whenever possible, on the pattern of performance on subscales of the Differential Ability Scale (DAS; Elliott, 1990), a standardized measure of cognitive abilities. When the DAS could not be administered, phenotypic assignment was based on performance on subscales of the Wechsler Adult Intelligence Scale-Revised (WAIS-R; Wechsler, 1981), the Developmental Test of Visual Motor Integration (VMI; Beery, 1989) or the Mental Scale of the Bayley Scales of Infant Development (Bayley, 1969; Bayley, 1993). Use of the Bayley to determine if a child's cognitive profile is consistent with the WSCP has been validated in a study comparing very young children with WS to very young children with Down syndrome (Mervis and Bertrand, in press). In the present study, the Bayley measure was used for only one child (K1895 II-4), who was 15 months old at the time of assessment. Quantitative data resulting from these tests were used to test for the presence of the WSCP, which involves weakness on the pattern construction subtest and strength

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on the digit recall subtest relative to performance on other subtests. The results of these studies are summarized in Tables 1-3.

To determine the sensitivity of the WSCP assessment, the DAS was also administered to 48 individuals with WS ranging in age from 4 to 47 years (IQ range 35-84). Of these  
 5 individuals, 45 fit the WSCP; 40 had an excellent fit, 3 had a very good fit, and 2 had a good fit. To determine specificity, the performance of 25 control individuals with below-average IQ (IQ range 30-95) was also examined. Some of these controls had other syndromes (e.g., Down syndrome or Fragile X syndrome); the others had no specific diagnosis. Of these individuals, 23  
 10 of 25 definitely did not fit the WSCP. Thus, the WSCP measure has excellent sensitivity (.94) and specificity (.92).

The WS personality was assessed by examining individuals for seven personality characteristics commonly found in WS. Standardized assessments of personality could not be used because these methods do not address the unique characteristics included in the WS personality. Individuals who showed at least 4 of 7 of the characteristics were considered to  
 15 have the WS personality. Individuals who showed 3 characteristics were classified as uncertain. Individuals who showed 2 or fewer characteristics were considered not to have the WS personality. To determine the sensitivity and specificity of our measure, we evaluated 85 individuals with WS and a control group of 65 individuals with mental retardation or borderline normal intelligence. Eighty-three of 85 WS individuals had the WS personality.

20

Table 1

Phenotypic evaluation of individuals with partial WS phenotype and control subjects

	<u>Individual</u>	<u>SVAS</u>	<u>Facies</u>	<u>WSCP</u>	<u>WSP</u>	<u>MR/DD</u>	<u>DEL</u>
25	K1895						
	I-2	+	3	+	-	-	D (~300kb)
	I-3	-	0	-	-	-	N
	II-1	-	0	-	-	-	N
	II-2	+	5	+	-	-	D (~300 kb)
30	II-3	-	0	-	-	-	N
	II-4	+	2	+	-	-	D (~300 kb)
	II-5	-	0	-	-	-	N
	II-6	-	0	-	-	-	N
	K2049						
35	I-1	+	4	+	-	-	D (83.6 kb)
	II-2	+	2	+	-	-	D (83.6 kb)
	II-3	-	2	+	-	-	D (83.6 kb)

Table 1 (continued)

Phenotypic evaluation of individuals with partial WS phenotype and control subjects

	<u>Individual</u>	<u>SVAS</u>	<u>Facies</u>	<u>WSCP</u>	<u>WSP</u>	<u>MR/DD</u>	<u>DEL</u>
5	K2049 (continued)						
	II-4	+	0	+	-	-	D (83.6 kb)
	II-5	-	0	-	-	-	N
	II-6	+	4	+	-	-	D (83.6 kb)
10	II-7	-	0	-	-	-	N
	III-1	-	0	-	-	-	N
	III-2	-	0	+	-	-	D (83.6 kb)
	III-3	+	0	U	-	-	D (83.6 kb)
	III-4	-	0	-	-	-	N
15	III-5	-	0	-	-	-	N
	III-6	+	0	+	-	+	D (83.6 kb)
	III-7	+	0	-	-	-	D (83.6 kb)
	III-8	-	0	-	-	-	N
	IV-1	-	0	-	-	-	N
20	IV-2	+	6	+	-	-	D (83.6 kb)
	Classic WS						
	13759	+	13	+	6	+	D (>500 kb)
	13946	+	16	+	+	+	D (>500 kb)
	14033	+	15	+	+	+	D (>500 kb)
25	14101	+	13	+	+	+	D (>500 kb)
	14576	-	14	+	+	+	D (>500 kb)
	15083	+	13	+	+	+	D (>500 kb)
	15266	+	13	+	+	+	D (>500 kb)
	17402	+	13	+	+	+	D (>500 kb)
30	18031	-	14	+	+	+	D (>500 kb)
	18296	+	14	+	+	+	D (>500 kb)
	Autosomal Dominant SVAS						
	12903	+	1	-	0	-	N
	12905	+	3	-	0	-	N
35	12906	+	2	-	0	-	N
	12907	+	0	-	0	-	N
	13222	+	1	-	-	-	N
	13835	+	0	-	-	-	N
	14104	+	1	-	0	-	N
40	14107	+	0	-	0	-	N
	17607	+	2	-	0	-	N
	20583	+	2	-	-	-	N

Table 1. Phenotypic evaluation was completed in members of two families with a partial WS phenotype (K1895 and K2049), individuals with classic WS, and individuals with autosomal dominant SVAS resulting from *ELN* mutations. Phenotypic assignments included the presence (+) or absence (-) of SVAS, specific WS cognitive profile (WSCP), and mental retardation or

developmental delay (MR/DD). Individuals were assigned 0-7 of 7 possible WS personality characteristics (WSP); individuals were considered affected if they had  $\geq 4$  characteristics and unaffected if they had  $\leq 2$  characteristics. The number of WS facial features present (Facies) is also indicated (0-16 of 16 possible WS facial features). The phenotypic assessments for WSCP were based on numerical scores obtained from one of the following standardized tests: 1) Differential Ability Scales; 2) Wechsler Adult Intelligence Scale-Revised; or 3) Mental Scale of the Bayley Scales of Infant Development. Individual III-3 of K2049 was characterized as phenotypically uncertain (U) with respect to WSCP because of a seizure disorder treated with anti-convulsant medication. Individual III-6 had mild developmental delay, with an IQ=64; the 95% confidence interval was 58-71 (an IQ score of  $\geq 70$  would be in the normal range). The presence (D) or absence (N) of a chromosome 7q11.23 deletion is indicated at right. Note that SVAS, mild WS facial features, and the WSCP cosegregated with deletions in K1895 and K2049. Incomplete penetrance and variable expression were apparent in these kindreds.

Table 2  
Assessment of WSCP for individuals completing the DAS

	Individual	PC $\geq$ T	Exclusion		Strength of Fit to WSCP		
			PC $\geq$ D	PC $\geq$ 20%	D $\geq$ T	V>PC	TOTAL
20	K1895						
	I-2				2	2	4
	I-3		x				
	II-1	x	x				
25	II-2				2	2	4
	II-3	x		x			
	II-5	x		x			
	II-6	x	x	x			
	K2049						
30	III-1	x		x			
	III-2				2	2	4
	III-4	x					
	III-5		x				
	III-6				2	2	4
35	III-7		x				
	III-8	x	x				
	IV-1			x			
	IV-2				2	2	4
	Classic WS						
40	13759				2	2	4
	13946				2	2	4
	14033				2	2	4
	14101				2	2	4
	14576				2	2	4
45	15083				2	2	4
	15266				2	2	4

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Table 2 (continued)  
Assessment of WSCP for individuals completing the DAS

	<u>Individual</u>	<u>Exclusion</u>			<u>Strength of Fit to WSCP</u>		
		<u>PC≥T</u>	<u>PC≥D</u>	<u>PC≥20%</u>	<u>D&gt;T</u>	<u>V&gt;PC</u>	<u>TOTAL</u>
5	Classic WS (continued)						
	17402				2	2	4
	18031				2	2	4
10	18296				2	2	4
	Autosomal Dominant SVAS						
	12903	x	x	x			
	12905	x	x	x			
	12906			x			
15	12907	x		x			
	13222	x	x	x			
	13835	x	x	x			
	14104	x	x	x			
	14107	x	x	x			
20	17607	x	x	x			
	20583	x	x	x			
	Normal						
	29998	x		x			
	29999		x	x			

25 Table 2. WSCP evaluation using the DAS was completed in members of K1895, K2049, autosomal dominant SVAS, normal controls, and individuals with classic WS. DAS evaluation included assessment of pattern construction (PC), digit recall (D), verbal abilities (V), and mean standard score for the core subtests (T). The WSCP was excluded if PC≥T, PC≥D, or PC≥20th percentile. For individuals who were not excluded, level of fit to the WSCP was based on total score: 0-1 point = poor fit; 2 = good fit; 3 = very good fit; 4 = excellent fit.

Table 3  
Assessment of WSCP for individuals who did not complete the DAS

35	INDIVIDUALS WHO COMPLETED THE WAIS-R					
		Exclusion		Inclusion		
	<u>Individual</u>	<u>PC≥D</u>	<u>PC≥20%</u>	<u>D&gt;PC</u>	<u>V&gt;PC</u>	<u>WSCP</u>
	K2049					
40	I-1			◇	+	+
	II-2			+	+	+
	II-3			+	+	+
	II-4			+	+	+
	II-5	+				-
45	◇ Digit recall assessment was inappropriate, due to dementia					

Table 3 (continued)  
Assessment of WSCP for individuals who did not complete the DAS

INDIVIDUALS WHO COMPLETED THE VERBAL WAIS-R AND THE VMI			
	Exclusion	Inclusion	
<u>Individual</u>	<u>VMI AE &gt; 10 years</u>	<u>Verbal WAIS-R &gt; VMI</u>	<u>WSCP</u>
K2049			
II-6		+	+
II-7	+		-

INDIVIDUAL WHO COMPLETED THE BAYLEY			
	Bayley I	Bayley II	
<u>Individual</u>	<u>%LI &gt; %NLI</u>	<u>%LI &gt; %NLI</u>	<u>WSCP</u>
K1895			
II-4	+	+	+

Table 3. Adults who could not complete the DAS were phenotypically characterized with the Wechsler Adult Intelligence Scale-Revised (WAIS-R) whenever possible. Phenotypic characterizations based on the WAIS-R included assessments of pattern construction (PC; block design subtest), digit recall (D), and verbal abilities (V). Inclusion criteria for Bayley I and Bayley II were based on passing a greater proportion of language items attempted (%LI) than non-language items attempted (%NLI). Individuals II-6 and II-7 of K2049 only completed the verbal portion of the WAIS-R, so additional characterization was completed using the Developmental Test of Visual Motor Integration (VMI). VMI AE = age equivalent for the VMI. Individual II-4 of K1895 was too young to complete the DAS, so phenotypic characterization was carried out using the Bayley test.

Sixty-four out of 65 control individuals did not have the WS personality. Thus, the WS personality measure had a sensitivity of 0.98 and a specificity of 0.98.

Phenotypic characterization of individuals with isolated, autosomal dominant SVAS indicated that these individuals did not manifest the other major features of WS (Table 1 and data not shown). Occasionally, an individual with autosomal dominant SVAS presented with a few WS facial features ( $\leq 6$  of 16) and/or a hernia, but no other WS phenotypic characteristics were observed. In particular, no one with autosomal dominant SVAS showed evidence of the WSCP. Because these individuals each harbors a mutation (translocation or point mutation) that disrupts one *ELN* allele, the data indicate that *ELN* mutations cause vascular disease but not impaired visuospatial constructive cognition.

Continued ascertainment and phenotypic characterization revealed two families with a partial WS phenotype (Figures 1A and 1B). Most affected members of these families had

SVAS, some WS facial features, and the WSCP. These individuals showed levels of verbal ability and auditory short-term memory similar to those of unaffected family members, but their visuospatial constructive abilities were markedly impaired. Affected members lacked other features of WS, including the WS personality and mental retardation (Table 1). Serum calcium levels during infancy were available for only four individuals, but none showed evidence of hypercalcemia (data not shown). No WS phenotypic characteristics were present in unaffected family members.

Previous studies indicate marked intra- and inter-familial variability of expression and incomplete penetrance for autosomal dominant SVAS (Curran et al, 1993; Ewart et al., 1993b; Morris et al., 1993; Ewart et al., 1994). Similar variability was found in individuals with partial WS phenotypes. For example, SVAS was severe and required surgery in two members of K2049 (individuals III-3 and III-7) and had led to early death in three members of K1895 (individuals not shown on pedigree). Other affected members of these kindreds exhibited mild to moderate SVAS, and vascular disease was not clinically apparent in two members of K2049 (individuals II-3 and III-2). Some WS facial features (2-6 of the 16 possible facial characteristics associated with classic WS) were observed in all affected members of K1895 and in 5 of 10 affected members of K2049, but these features did not fulfill the diagnostic criteria for WS ( $\geq 9$  of 16 facial features). WSCP was observed in all affected members of K1895 and in 8 of 10 affected members of K2049; one member of K2049 did not fulfill the diagnostic criteria for WSCP (individual III-7) and one individual (III-3) was classified as uncertain. These phenotypic studies indicate autosomal dominant co-inheritance of SVAS, some WS facial features, and WSCP in two families with variable phenotypic expression and incomplete penetrance. Identification of individuals with a partial WS phenotype supports the hypothesis that WS is a contiguous gene deletion syndrome.

## EXAMPLE 2

### Association of partial WS phenotypes with submicroscopic chromosome 7q11.23 deletions

If WS is a contiguous gene deletion syndrome, individuals with a partial WS phenotype should have smaller deletions in the chromosome 7q11.23 region than those seen with classic WS. To test this hypothesis, a partial physical map of the region deleted in WS was constructed. Because *ELN* is completely deleted in individuals with classic WS, these experiments were

initiated by isolating and characterizing *ELN* genomic clones. These clones were used for genomic walking into regions flanking *ELN*. A set of contiguous cosmid clones generated by walking 3' of *ELN* is shown in Figure 2. Attempts to extend the cloned coverage in a direction 5' of *ELN* using phage, cosmid, P1, P1 artificial chromosomes and yeast artificial chromosome (YAC) libraries were less successful; very few clones were isolated from this region and clones that were isolated were unstable. Clones were characterized by restriction enzyme analyses and placed on the physical map by somatic cell hybrid Southern analyses or sequence-tagged-site mapping by means of the polymerase chain reaction (PCR). These clones span ~350 kb of chromosome 7q11.23, including the entire *ELN* locus. No other genes were previously mapped to this region.

To determine if individuals with a partial WS phenotype carried deletions involving chromosome 7q11.23, fluorescence *in situ* hybridization (FISH) was performed using cosmids that span the *ELN* locus. All affected members of K1895 showed *ELN* hemizyosity, while unaffected members had two *ELN* alleles (Fig. 1A). Additional FISH analyses revealed hemizyosity with probes c138-13c, c1-4a2, 106G5, and 135F3, but not with 157F3, 39E7, and 198G11 (data not shown). These results indicated that affected members of K1895 harbor a chromosome 7q11.23 deletion that includes *ELN* and extends through the locus corresponding to cosmid 135F3 (Fig. 2). Additional FISH analyses using YACs from this region are consistent with these data and indicate a deletion of approximately 300 kb (unpublished data). By contrast, FISH analyses of individuals with classic WS showed hemizyosity with all clones tested, suggesting that these deletions span more than 500 kb (unpublished data).

A deletion associated with SVAS in two members of K2049 (Ewart et al., 1994) was previously described. This deletion disrupted *ELN*, beginning in intron 27 and extending 3' of the gene. Oligonucleotides flanking the deletion breakpoints were used to define a novel PCR product of 403 bp in all phenotypically affected members of this kindred (Fig. 1B). This product was not seen in unaffected members. Physical mapping and restriction analyses indicated that the deletion had removed ~85 kb of genomic DNA (Fig. 2), a much smaller region than is missing in individuals with classic WS. These data indicate that a partial WS phenotype, including SVAS, some WS facial features, and WSCP, cosegregates with the ~85 kb deletion in this family. Because intragenic mutations of *ELN* cause isolated SVAS and some WS facial features (Curran et al., 1993; Morris et al, 1993; Olson et al, 1995), but not the WSCP (Table 1),



a gene responsible for the impaired visuospatial constructive cognition must be located immediately 3' of *ELN*.

### EXAMPLE 3

#### Identification of a Protein Kinase Immediately 3' of *Elastin*

To screen for a gene that contributes to impaired visuospatial constructive cognition, cosmids cELN-11d, c138-13c, and c1-4a2 were used in cDNA screening analyses, but no genes were identified. The specific hypothesis that hemizyosity of a gene encoding a protein kinase could cause the impaired visuospatial constructive cognition was also tested. This hypothesis was based on observations that targeted disruption of genes encoding protein kinases results in mice with impaired spatial learning (Grant et al., 1992; Abeliovich et al., 1993a, Abeliovich et al., 1993b). Oligonucleotides complementary to sequences conserved in tyrosine kinases were designed and PCR analyses were performed with genomic clones from the physical map. A specific product of 315 bp was identified from cosmid c138-13c. This PCR product was cloned; DNA sequence analyses revealed an open reading frame of 113 nucleotides with complete homology to *LIM-kinase1* (*LIMK1*), a recently identified gene encoding a protein kinase with LIM domains (Mizuno et al., 1994; Bernard et al., 1994). Oligonucleotides based on published cDNA sequences were used in PCR experiments to clone *LIMK1* cDNA from a human hippocampal cDNA library. PCR analyses of DNA from somatic cell hybrids, cosmids, P1s, and YACs localized *LIMK1* to the deleted region on chromosome 7q11.23. These data place *LIMK1* immediately 3' of *ELN* and within the ~85 kb deletion identified in K2049.

Oligonucleotides based on published cDNA sequences were used in PCR experiments to clone a *LIMK1* cDNA from a human hippocampal library (*LIMK1* nucleotides 96-2039). A human hippocampal cDNA library (catalog #936205, Stratagene), was plated at a density of  $5 \times 10^4$  pfu/15 cm plate to obtain  $1 \times 10^6$  total pfu. Duplicate filters were probed with cELN-11d, c138-13c, and c1-4a2, which had been radiolabeled to a high specific activity ( $>1.0 \times 10^9$  cpm/ $\mu$ g DNA) using random hexamer priming as described by Feinberg and Vogelstein (1984). *LIMK1* cDNA fragments were obtained from the same hippocampal cDNA library using PCR with *rTth* DNA polymerase and various primers designed from the published *LIMK1* cDNA sequence (Mizuno et al., 1994). The open reading frame (*LIMK1* nucleotides 93-1936) was

amplified and cloned using the following primers: 5'-ATGAGGTTGACGCTACTTTGTTGC-3' (SEQ ID NO:1) and 5'-TCAGTCGGGGACCTCAGGGTGGGC-3' (SEQ ID NO:2).

PCR primers were designed to amplify the region of homology in the kinase domains of PDGF receptor, HER2, HER3, FGF-FLG, FGF-BEK, insulin receptor, and IRR (sequences  
5 obtained from Genbank). The primers used were 5'-GACTTTGGGCTGGCTCGAGACATG C-3' (SEQ ID NO:3) and 5'-CTCCGGAGCCATCCACTTGACTGGC-3' (SEQ ID NO:4). PCR conditions were one cycle of 94°C for 10 min, followed by 30 cycles of 94°C for 1 min, 49°C for 1 min, and 72°C for 1 min, ending with one cycle of 72°C for 10 min. Clones c138-3c, cELN-11d, and c138-13c were used as templates. Products were cloned into pBluescript II SK-  
10 (Stratagene) using standard T/A cloning technology (Marchuk et al., 1991) and sequenced.

Genomic clones were obtained from the following sources: c138-3c,  $\lambda$ 4,  $\lambda$ 5, cELN-11d, and c138-13c were derived from primary cosmid and phage libraries constructed earlier in our laboratory (Curran et al., 1993; Ewart et al., 1994). Cosmids cos6 and c1-4a2 were obtained from an amplified placental library (Stratagene). Cosmids 129F9, 128F2, 106G5, 135F3, 157F3,  
15 39E7, and 198G11 were isolated from the chromosome 7-specific flow-sorted cosmid library constructed at the Lawrence Livermore National Laboratories.

#### DNA Sequence Analyses and Testing of Putative Coding Regions

Cycle sequencing with oligonucleotides generated from the *LIMK1* cDNA sequence and from our DNA sequence analyses was used to define the structure of *LIMK1* using cosmids  
20 cELN-11d, c138-13c, and c1-4a2. Cycle sequencing of cosmids was performed using 1.5 pmol of primer, 15 fmol of template, and the dsDNA Cycle Sequencing System (GibcoBRL). Reaction conditions were 94°C for 3 min, 20 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, 10 cycles of 94°C for 30 s and 72°C for 1 min. Cycle sequencing products were electrophoresed on 6% denaturing polyacrylamide gels (National Diagnostics) the same day the  
25 reactions were performed. Also, the addition of formamide to a final concentration of 4% allowed cycle sequencing of regions that could not be sequenced by standard conditions.

Sanger sequencing was performed using the Sequenase v2.0 DNA Sequencing Kit (United States Biochemical) under standard conditions. Sequence analysis relied on the IG software package and the BLAST network service from the National Center for Biotechnology  
30 Information.

The intron-exon structure and predicted amino acid sequences are shown in Table 4 and Figure 3. *LIMK1* is composed of 16 exons, spans 37 kb, and is located 15.4 kb 3' of *ELN* (Fig.

- 2). Predicted amino acid sequence analyses revealed two putative LIM domains (amino acids 25-75 for LIM-1, 84-137 for LIM-2; Way and Chalfie, 1988; Freyd et al., 1990; Karlsson et al., 1990), a Dlg homology region (DHR; amino acids 165-258; Ponting, 1995), a possible PEST domain (PESTFIND score = 6.3; amino acids 264-289; Rogers et al., 1986), a kinase domain (amino acids 345-594), and a putative nuclear localization signal (NLS; amino acids 499-506; Forbes, 1992). Comprehensive DNA sequence analyses confirmed the location and structure of *LIMK1*. Together, these data place *LIMK1* immediately 3' of *ELN* and within the ~85 kb deletion identified in K2049.
- 5

Table 4  
*L/MK1* genomic structure

Exon #	Intron	Exon Size (55) <sup>a</sup>	Intron	Exon Size (55) <sup>a</sup>
1	actcccttccaccctgcagGAAGCGAGTT	(SEQ ID NO: 5)	GGAGAGGAAGTgcgcgggcccgcggggcgc	(SEQ ID NO: 6)
2	gcccggccctctcctgcagGTGTGTGAC	(SEQ ID NO: 7)	ACTGCTTCAGTtaggtgggtgccccaggg	(SEQ ID NO: 8)
3	cctcctcaccctccaccagGTGGCTGGGG	(SEQ ID NO: 9)	AGCTGTACTGgtgagcgcctcctgcttgc	(SEQ ID NO: 10)
4	caccccggggctcttgacCGGGCACTGC	(SEQ ID NO: 11)	AGCTGTACTGgtgagcgcctcctgcttgc	(SEQ ID NO: 12)
5	gacccctgcttaccacagAGTGGATCCG	(SEQ ID NO: 13)	GCGTCCAGGGgtgagtgcccgccctgccga	(SEQ ID NO: 14)
6	cacatgcctgctgtcccccagATTGACTTGC	(SEQ ID NO: 15)	CCTGGACGAGgtacggtcctgagctctgg	(SEQ ID NO: 16)
7	gcacatgtgtgtccccccagGAGGAGCTGC	(SEQ ID NO: 17)	AACCTGTCTTgttaagtccagcctgctcctcg	(SEQ ID NO: 18)
8	cctctgtgtccacacgcagGTGACACACC	(SEQ ID NO: 19)	GGCTATCAAGgtacagagcatgccagggtc	(SEQ ID NO: 20)
9	gcctgtgtgtgtccccccagGTGAAGTTCA	(SEQ ID NO: 21)	CCTCAAGGAGgtcagtgagcggaatgcct	(SEQ ID NO: 22)
10	ccattcttctccatcccccagGACAGCCAGT	(SEQ ID NO: 23)	CAAGAGCATGgtgagtcctgggcagagcca	(SEQ ID NO: 24)
11	tcccgtgtccccgtcccttagGCTTACTTCC	(SEQ ID NO: 25)	ATCAGGGATGgtgagtgagccgggtgctct	(SEQ ID NO: 26)
12	acccggtttcaccttcccccagAACAGGATG	(SEQ ID NO: 27)	GGTCCGCGAGgtgagtagccaggccccacg	(SEQ ID NO: 28)
13	cagtcggtctcttattccagGCGGCACTTA	(SEQ ID NO: 29)	ATGATCAACGgttagtggttcagccctgccc	(SEQ ID NO: 30)
14	ccgggcttgtactggacagATCATCGGGC	(SEQ ID NO: 31)	CCTGTGCGAGgttaggtccagggttggttag	(SEQ ID NO: 32)
15	cccaccacctgtcaccacagGCCATCCTTT	(SEQ ID NO: 33)	CCGAGAAAGAGgtgagtggtgggtgggcccctg	(SEQ ID NO: 34)
16		(SEQ ID NO: 35)	CCCCGACTGA . . . (SEQ ID NO: 36)	

Table 4. DNA sequence analyses of genomic clones containing *L/MK1* indicate that this gene consists of 16 exons and spans ~37 kb. DNA sequences for intron/exon boundaries are shown using the consensus sequences defined by Shapiro and Senapathy (1987). Intronic sequences are shown in lower-case, exon sequences in upper-case, bold, and underlined. The number of nucleotides for each exon is in parentheses. <sup>a</sup>The number of coding nucleotides (rather than the total number of nucleotides) is indicated for the first and last exons. Exon 16 extends 1223 bp beyond the stop codon to the polyadenylation site.

### Cosegregation of LIMK1 Hemizygosity and Impaired Visuospatial Constructive Cognition

To test the hypothesis that *LIMK1* hemizygosity contributes to the WSCP, FISH analyses were performed with metaphase chromosomes from individuals with both partial and classic WS phenotypes using cosmids cELN-11d, c138-13c, and c1-4a2. Cosmid probes c138-13c and c1-4a2 were labeled with biotin using a nick translation kit (GibcoBRL). Metaphase chromosome spreads were prepared from EBV transformed lymphoblastoid cell lines derived by standard procedures of colcemid arrest, hypotonic treatment and acetic acid-methanol fixation. Slides were prepared as described by Lichter et al. (1988) and hybridized with a probe mixture containing c138-13c, c1-4a2, human C<sub>0</sub>t-1 DNA, and a chromosome 7-specific alpha satellite cocktail (Oncor, Inc.). For other hybridizations, cosmids 135F3, 157F3, 39E7, and 198G11 were used. Following overnight hybridization and subsequent washing, slides were incubated with streptavidin-Cy3 (cosmids) and anti-digoxigenin FITC (chromosome 7 marker). Slides were counterstained with DAPI/Antifade (Oncor, Inc.). Metaphases were scored using an epifluorescence Olympus PX50 microscope with a triple band pass filter, and then captured using a cooled CCD camera and imaging system designed specifically for FISH (Oncor, Inc.).

*LIMK1* was completely deleted from one chromosome 7 homologue in affected members of K1895 and K2049 and in 62 of 62 individuals with classic WS (e.g., Fig. 4A-4C). *LIMK1* was not deleted in 6 of 6 individuals with isolated and *de novo* SVAS who showed some WS facial features (e.g., Fig. 4D). *LIMK1* hemizygosity was not observed among more than 100 control individuals (Fig. 4E and data not shown). These data indicate that *LIMK1* is deleted in individuals with classic and partial WS but not in individuals with isolated SVAS, and suggest that *LIMK1* hemizygosity contributes to the WSCP.

### EXAMPLE 4

#### Direct DNA sequence analysis of the ~85 kb deletion region reveals only *LIMK1* and *ELN*

To determine if *LIMK1* is the only gene from this region likely to contribute to cognitive development, the ~85 kb segment deleted in K2049, along with flanking sequences, was sequenced. Cycle sequencing and Sanger sequencing were performed as described above. Four cosmids and two phage (cos6,  $\lambda$ 4,  $\lambda$ 5, cELN-11d, c1-4a2, and 129F9) that form an overlapping contig of the entire 83.6 kb deletion region in K2049 and the flanking sequences surrounding the breakpoints were sequenced. A modification of the sequencing procedure described by Mardis (1994) was used. Approximately 900 single-stranded M13 clones were sequenced for each cosmid using dye-primer chemistry (Applied Biosystems, Epicentre Technologies, and

Amersham). Products from the sequencing reactions were run on either an ABI 373a Stretch DNA Sequencer or an ABI 377 Prism DNA Sequencer. The sequence data were processed using the XGAP algorithms (Dear and Staden, 1991; Dear and Staden, 1992). Gaps in the 83.6-kb contig were filled in by one of the following methods: 1) direct sequencing of cosmids using specific primers; 2) sequencing of PCR products generated using primers that flank the gaps; or 3) subcloning restriction fragments containing the gaps into pBluescript II SK<sup>+</sup> (Stratagene) and sequencing them using dye-primers.

The 83.6-kb sequence was analyzed for known genes using GENQUEST and BLAST servers. Potential coding exons, polyadenylation sites, and CpG islands were identified by versions 1.2 and 2 of the GRAIL neural network. All putative coding regions with either excellent or good scores were tested for mRNA expression by either Northern-blot analysis (human MTN blot 1 and human fetal MTN blot) or a combination of Northern-blot analysis and RT-PCR.

RT-PCR was performed according to manufacturer's instructions using 200 ng of total RNA and the ThermoStable *rTth* Reverse Transcriptase RNA PCR kit (Perkin Elmer). Controls included 100 ng of genomic DNA, 100 ng of genomic DNA that had been digested with 10 units of DNase I, and a water blank. RNA samples were prepared with and without DNase I treatment. Reverse transcription was performed for 15 minutes at 60°C. PCR was performed for either 35 or 50 cycles on a Perkin Elmer 9600 GeneAmp PCR System using the following cycling conditions: 1) initial denaturation at 94°C for 3 minutes; 2) subsequent denaturation at 95°C for 10 seconds; 3) annealing and extension at 60°C for 15 seconds. Products were electrophoresed through a 5% 3:1 agarose gel (FMC) and visualized by staining with ethidium bromide.

DNA sequence analyses defined two ordered contigs of 41,566 and 65,607 base pairs. These contigs were separated by a gap of approximately 250 base pairs (Figure 2). Due to its high GC content, this gap could not be sequenced using primer walking, amplified PCR products, or subcloning. The restriction maps predicted from DNA sequence analyses were identical to maps generated using BamHI, EcoRI, and HindIII. The size of the deletion was 83.6 kb. The sequences were analyzed for the presence of known genes using the GRAIL, GENQUEST, and BLAST servers (Shah et al., 1994; Altschul et al., 1990). Only *ELN* and *LIMK1* were detected.

Comparison between the cDNA and genomic sequence revealed 16 *LIMK1* exons that span 37 kb of genomic DNA. Sequence analyses also indicated that *LIMK1* is located 15.4 kb 3' of *ELN* (Figure 2). Predicted amino acid sequence analyses identified all previously described domains including LIM-1, LIM-2, a Dlg homology region, a putative nuclear localization signal, and a kinase domain (Mizuno et al., 1994; Ponting, 1995). In addition, sequence analyses revealed a possible PEST domain (PESTFIND score = 6.3; amino acids 264-289; Rogers et al., 1986).

Sequences were also scanned for potential coding regions using versions 1.2 and 2 of the GRAIL neural network (Table 5). Except for *ELN* (GRAIL identified 16 of 30 exons) and *LIMK1* (15 of 16 exons), no other putative exons categorized as excellent were identified by GRAIL. Additionally, GRAIL identified seven possible coding sequences categorized as good (six within the 83.6 kb deletion region) and eleven categorized as marginal. All possible coding sequences classified as good were tested using either multiple-tissue Northern analyses or a combination of Northern analyses and reverse transcription-PCR of total RNA extracted from fetal and adult human brain (Table 5 and data not shown). No evidence for expression of these additional possible coding sequences was found.

A remarkable finding of DNA sequence analyses was the high density of Alu repetitive elements in the 83.6 kb deletion region. A total of 120 full or partial Alu sequences was identified, for an average density of ~1.4/kb. This is 6-fold more than the estimated average density of 0.25/kb (Hwu et al., 1986; Slightom et al., 1994). One partial LINE sequence and one MER14-like element were also identified, as well as three large d(CA)- repeats (Figure 2). One of the d(CA)-repeats had been previously identified (Foster et al., 1993). Sequence analyses also defined the breakpoints for the K2049 deletion; both breakpoints consisted of Alu repeats, suggesting that a recombination event between these Alu sequences may have been responsible for the deletion.

Table 5

GRAIL Analyses of DNA Sequences within the 83.6 kb Deleted Region

<u>Putative Coding Region</u>	<u>Size (bp)</u>	<u>Grail Version</u>	<u>Grail Quality</u>	<u>Strand (F/R)</u>	<u>Exclusion</u>
ELN-29	60	1.2	E	F	-
ELN-30	75	1.2,2	E	F	-
208pr3	114	1.2	G	R	N,RP
124pr3	85	1.2	G	R	N,RP
90pr1	123	1.2	G	R	N,RP
LIMK-2	97	1.2,2	E	F	-
441pr1	141	1.2,2	G	F	N,RP
LIMK-3	139	1.2,2	E	F	-
LIMK-4	110	1.2,2	E	F	-
LIMK-5	207	1.2,2	E	F	-
LIMK-6	106	1.2,2	E	F	-
LIMK-7	167	1.2,2	E	F	-
LIMK-8a	36	2	E	F	-
LIMK-8b	123	1.2,2	E	F	-
LIMK-9	87	1.2,2	E	F	-
LIMK-10	132	1.2,2	E	F	-
LIMK-11	60	2	G	F	-
LIMK-12	66	1.2,2	E	F	-
604pr2	39	2	G	F	N,*
LIMK-13	157	1.2,2	E	F	-
LIMK-14	56	1.2,2	G	F	-
LIMK-15	158	1.2,2	E	F	-
LIMK-16	163	1.2,2	E	F	-
604pr3	31	2	G	R	N,*

Table 5. Only the putative coding regions with either excellent or good scores are listed in this table. The putative coding regions are either named after the gene and exon number (e.g., ELN-29 is exon 29 of the *elastin* gene) or given an assigned name (e.g., 208pr3). Putative exons are given either excellent (E) or good (G) scores. F = forward strand in relation to *ELN* and *LIMK*; R = reverse strand. N = no evidence for expression by Northern blot analysis; RP = no evidence for expression by RT-PCR; \* = putative coding region not tested by RT-PCR because the coding region was too short; - = not tested because the putative coding region is an exon of a known gene.

EXAMPLE 5*LIMK1* and *ELN* Expression in the Developing Brain

To determine the expression pattern of *LIMK1*, Northern analyses were performed with mRNA extracted from fetal and adult tissues. Northern blots containing ~2 µg/lane of poly(A)<sup>+</sup>



mRNA were purchased from Clontech (human MTN blot 1, human brain blots 2 and 3, human fetal MTN blot, and a mouse MTN blot). The blots were hybridized in ExpressHyb solution (Clontech) according to the manufacturer's instruction, with either <sup>32</sup>P-end-labeled *LIMK1* oligonucleotide probe (704-742 bp) or *LIMK1* (104-2038 bp), *ELN* (1-1123 bp), and  $\beta$ -*actin* cDNA clones that had been radiolabeled using random hexamer priming (Feinberg and Vogelstein, 1984). Each *LIMK1* Northern blot was analyzed by phosphorimage analyses (Molecular Dynamics) to determine the amounts of *LIMK1* RNA relative to  $\beta$ -*actin* mRNA.

A *LIMK1* oligonucleotide probe hybridized to a single mRNA of ~3.3 kb in all fetal and adult tissues examined (Fig. 5). Phosphorimage analyses indicated that mRNA levels varied considerably but were highest in both fetal and adult brain. Northern analyses of tissue from different regions of the adult human brain demonstrated that *LIMK1* is ubiquitously expressed, with mRNA levels highest in the cerebellum, caudate nucleus, substantia nigra, and the occipital pole (Fig. 5). Analyses of adult murine tissues indicated that *LIMK1* is most strongly expressed in testes and brain (data not shown). These data establish that *LIMK1* is widely expressed during fetal and adult life, but that *LIMK1* mRNA levels are highest in the brain.

*In situ* hybridization analyses of *LIMK1* expression in the embryonic human nervous system demonstrated that *LIMK1* is expressed in several discrete regions of the brain and spinal cord (Figure 6). *In situ* hybridization was performed on 6mm-thick, paraffin embedded sections of freshly prepared human embryos, which were obtained from the MRC-funded Human Embryonic Tissue Bank, Institute of Child Health, London. A digoxigenin-labeled 625-bp cRNA probe specific to the 3'-untranslated portion of *LIMK1* cDNA was used to avoid areas of homology with other genes encoding proteins containing LIM and kinase domains; similar results were obtained, however, in some sections hybridized with a cDNA probe covering the kinase region and some of the 3'-untranslated sequence. The *in situ* protocol was based on the detection of digoxigenin-labeled RNA by alkaline phosphatase-conjugated anti-DIG FAB fragments (Boehringer Mannheim), as previously described (Wilkinson, 1992; Birren et al., 1993). Brightfield microphotography was carried out with an Olympus BH-2 and Fujichrome 64T film.

Analyses of *LIMK1* expression in a Carnegie stage 20 (postovulatory day 50) human embryo revealed expression in the ependymal layer of the fourth ventricle, with a lower level of expression extending into the mantle layer. *LIMK1* was expressed in specific regions of the

brain, with notably high levels in the medial olivary nucleus. In the cerebellum, expression was seen again in ependymal layer. Staining also occurred in ependymal layer of the mesencephalon, which additionally contained many *LIMK1*-expressing cells in the mantle layer. In the spinal cord, *LIMK1* was expressed in a diffuse pattern dorsally, with single-cell staining ventrally. In the mid-area of the spinal cord, expression was again seen in ependymal and mantle layers. Within the peripheral nervous system, extensive expression of *LIMK1* was seen in spinal ganglia, in the fifth nerve ganglion, and in part of the inner ear.

To determine if *ELN* is expressed in the brain, Northern analyses were performed with mRNA extracted from fetal and adult tissues. *ELN* was strongly expressed in adult heart and pancreas and in fetal lung, but exhibited negligible expression in adult and fetal brain.

#### EXAMPLE 6

##### Distinguishing between SVAS, WSCP and WS

Supravalvular aortic stenosis (SVAS), Williams syndrome cognitive profile (WSCP) and Williams syndrome are inherited diseases which are related in that they involve a set of contiguous genes. Persons with mutations in the elastin gene but who are wild-type for *LIMK1* and do not have deletions 3' of *LIMK1* have SVAS. Persons who have mutations affecting both *elastin* and *LIMK1* (hemizyosity) but do not have deletions greater than about 300 kb 3' of the *ELN* gene are diagnosed as having WSCP. Persons who are mutated in both the *ELN* and *LIMK1* genes (and have one wild-type copy of each of these genes) and have a deletion of greater than 300 kb from the 3' end of the *LIMK1* gene in the 3' direction are diagnosed as having WS. One may conclude that SVAS is due to a mutation in or loss of a single gene (*ELN*), WSCP is a result of mutations in or loss of two genes (*ELN* and *LIMK1*), and WS results from mutations in or a loss of at least 3 genes (*ELN*, *LIMK1* and an unidentified gene or genes located on chromosome 7 greater than 300 kb 3' of *LIMK1*). It is possible to diagnose which disease a patient may have by use of chromosomal analysis. The complete sequence of the elastin and *LIMK1* cDNAs have been published (Indik et al., 1987; Fazio et al., 1988; Mizuno et al., 1994; Cheng and Robertson, 1995). Using the known nucleic acid sequences for these two genes it is possible to assay for mutations in these genes. This can be done by any desired technique such as by sequencing to determine the presence of mutations, especially the presence of deletions or translocations affecting the genes, or by *in situ* hybridization to determine

whether these genes are hemizygous or homo- or heterozygous. Using the knowledge of these two genes one can assay to determine if the patient has at least SVAS (i.e., loss of or mutation in at least *ELN*), or at least WSCP (loss of or mutation in both *ELN* and *LIMK1*). To determine whether an individual has WS it is helpful to examine the chromosome beyond the 3' ends of *ELN* and *LIMK1*. To date, all Williams syndrome patients analyzed have been found to have a major deletion in chromosome 7 which includes deletion of both the *ELN* (at least partially) and *LIMK1* genes as well as greater than another 300 kb 3' of the *LIMK1* gene. Patients who have deletions of 100 kb or smaller 3' of the *LIMK1* gene have been diagnosed as having WSCP but not WS. The use of probes to analyze for the extent of deletion of chromosome 7 in individuals can distinguish between WSCP and WS.

Figure 2 shows a map of chromosome 7 in the region of *ELN* and *LIMK1* with a series of overlapping cosmids covering this region. The range of coverage from c138-3c through 198G11 is approximately 350 kb. *In situ* hybridization with 135F3, for example, can be used to determine if there is a deletion of 100 kb or less 3' of *LIMK1*. If 135F3 hybridizes to both sets of chromosomes then the individual probably will not have WS since the deletion will be too small to delete the third, as yet unknown, gene which lies 3' of *ELN* and *LIMK1* and which must be mutated or deleted to cause WS. To date, all WS individuals have been found to have a deletion greater than 500 kb covering *ELN* and *LIMK1* and greater than 300 kb 3' of *LIMK1*. Furthermore, it has been seen that when a person does have a deletion in *ELN* there is a 99% chance that this is a major deletion of greater than 500 kb including *LIMK1* and the other gene or genes involved in WS. This means that the presence of a deletion in *ELN* in one chromosome is 99% indicative of the presence of WS.

While the invention has been disclosed in this patent application by reference to the details of preferred embodiments of the invention, it is to be understood that the disclosure is intended in an illustrative rather than in a limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended claims.

#### LIST OF REFERENCES

Abeliovich, A., Chen, C., Goda, Y., Silva, A. J., Stevens, C. F., & Tonegawa, S. (1993a). Modified hippocampal long-term potentiation in PKC $\gamma$  mutant mice. *Cell* 75, 1253-1262.

- Abeliovich, A., Paylor, R., Chen, C., Kim, J. J., Wehner, J. M., & Tonegawa, S. (1993b). PKC $\gamma$  mutant mice exhibit mild deficits in spatial and contextual learning. *Cell* 75, 1263-1271.
- Altschul, F. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215, 403-410.
- Bayley, N. (1969). Bayley Scales of Infant Development. (New York, NY: Psychological Corporation).
- Bayley, N. (1993). Bayley Scales of Infant Development (2nd ed.) (San Antonio, TX: Psychological Corporation).
- Beery, K. E. (1989). Developmental Test of Visual Motor Integration (3rd revision). (Cleveland, OH: Modern Curriculum Press).
- Bellugi, U., Wang, P. P., & Jernigan, T. L. (1994). Williams syndrome: An unusual neuropsychological profile. In *Atypical Cognitive Deficits in Developmental Disorders: Implications for Brain Function*. S.H. Broman & J. Grafman, eds. (Hillsdale, NJ: Erlbaum), pp. 23-56.
- Bernard, O., Ganiatsas, S., Kannourakis, G., & Dringen, R. (1994). *Kiz-1*, a protein with LIM zinc finger and kinase domains, is expressed mainly in neurons. *Cell Growth & Dif.* 5, 1159-1171.
- Birren, S. J., Lo, L., and Anderson, D. J. (1993). Sympathetic neuroblasts undergo a developmental switch in trophic dependence. *Development* 119, 597-610.
- Budarf, M. L., Collins, J., Gong, W., Roe, B., Wang, Z., Bailey, L. C., Sellinger, B., Michaud, D., Driscoll, D. A., and Emanuel, B. S. (1995). Cloning a balanced translocation associated with DiGeorge syndrome and identification of a disrupted candidate gene. *Nat. Genet.* 10, 269-278.
- Capruso, D.X., Hamsher, K., Benton, A.L. (1995). Assessment of visuocognitive processes. In Clinical Neuropsychological Assessment: A Cognitive Approach. R. L. Mupou and J. Spector (eds.) (New York: Plenum), pp. 137-183.
- Cheng, A. K., and Robertson, E. J. (1995). The murine LIM-kinase gene (*limk*) encodes a novel serine threonine kinase expressed predominantly in trophoblast giant cells and the developing nervous system. *Mech. Dev.* 52, 187-197.
- Curran, M. E., Atkinson, D. L., Ewart, A. K., Morris, C. A., Leppert, M. F., and Keating, M. T. (1993). The elastin gene is disrupted by a translocation associated with supravalvular aortic stenosis. *Cell* 73, 159-168.
- Curran, M. E., Splawski, I., Timothy, K. W., Vincent, G. M., Green, E. D., and Keating, M. T. (1995). A molecular basis for cardiac arrhythmia: *HERG* mutations cause long QT Syndrome. *Cell* 80, 795-803.
- Dear, S., and Staden, R. (1991). A sequence assembly and editing program for efficient management of large projects. *Nucl. Acids Res.* 19, 3907-3911.
- Dear, S., and Staden, R. (1992). A standard file format for data from DNA sequencing instruments. *DNA Seq.* 3, 99-105.

- Dilts, C. V., Morris, C. A., and Leonard, C. O. (1990). Hypothesis for development of a behavioral phenotype in Williams syndrome. *Am. J. Med. Genet. Suppl.* 6, 126-131.
- Elliott, C. D. (1990). *Differential Ability Scales*. (San Diego, CA: Harcourt Brace Jovanovich).
- Ensing, G. J., Schmidt, M. A., Hagler, D. J., Michels, V. V., Carter, G. A., and Feldt, R. H. (1989). Spectrum of findings in a family with nonsyndromic autosomal dominant supravalvular aortic stenosis: A Doppler echocardiographic study. *J. Am. Coll. Cardiol.* 13, 413-419.
- Ewart, A. K., Jin, W., Atkinson, D. L., Morris, C. A., & Keating, M. T. (1994). Supravalvular aortic stenosis associated with a deletion disrupting the elastin gene. *J. Clin. Invest.* 93, 1071-1077.
- Ewart, A. K., Morris, C. A., Atkinson, D. L., Jin, W., Sternes, K., Spallone, P., Stock, D., Leppert, M., and Keating, M. T. (1993a). Hemizygoty at the elastin locus in a developmental disorder, Williams syndrome. *Nat. Genet.* 5, 11-16.
- Ewart, A. K., Morris, C. A., Ensing, G. J., Loker, J., Moore, C., Leppert, M., and Keating, M. T. (1993b). A human vascular disorder, supravalvular aortic stenosis, maps to chromosome 7. *Proc. Natl. Acad. Sci. USA* 90, 3226-3230.
- Fazio, M. J., Olsen, D. R., Hauh, E. A., Baldwin, C. T., Indik, Z., Ornstein-Goldstein, N., Yeh, H., Rosenbloom, and Uitto, J. (1988). Cloning of Full-length Elastin cDNAs from a Human Skin Fibroblast Recombinant cDNA Library: Further Elucidation of Alternative Splicing Utilizing Exon-specific Oligonucleotides. *J. Investigative Dermatology* 91:458464.
- Feinberg, A., and Vogelstein, B. (1984). A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 137, 266-267.
- Forbes, D. J. (1992). Structure and function of the nuclear pore complex. *Ann. Rev. Cell Biol.* 8, 495-527.
- Foster, K., Ferrell, R., King-Underwood, L., Povey, S., Attwood, J., Rennick, R., Humphries, S. E., and Henney, A. M. (1993). Description of a dinucleotide repeat polymorphism in the human elastin gene and its use to confirm assignment of the gene to chromosome 7. *Ann. Hum. Genet.* 57, 87-96.
- Freyd, G., Kim, S. K., and Horvitz, H. R. (1990). Novel cysteine-rich motif and homeodomain in the product of the *Caenorhabditis elegans* cell lineage gene *lin-11*. *Nature* 344, 876-879.
- Gilbert-Dussardier, B., Bonneau, D., Gigarel, N., Merrer, M. L., Bonnet, D., Philip, N., Serville, F., Verloes, A., Rossi, A., Ayme, S., Weissenbach, J., Mattei, M. G., Lyonnet, S., and Munnich, A. (1995). A novel microsatellite DNA marker at locus D7S1870 detects hemizygoty in 75% of individuals with Williams syndrome. *Am. J. Hum. Genet* 56, 542-544.
- Grant, S. G. N., O'Dell, T. J., Karl, K. A., Stein, P. L., Soriano, P., and Kandel, E. R. (1992). Impaired long-term potentiation, spatial learning, and hippocampal development in *fyn* mutant mice. *Science* 258, 1903-1910.
- Grimm, T. & Wesselhoeft, H. (1980). Zur genetic des Williams-Beuren syndrome und der isolierten form der supravalvularen aortenstenose: untersuchungen von 128 familien. *Z. Kardiol.* 69, 168-172.

- Hanks, S. K., Quinn, A. M., and Hunter, T. (1988). The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. *Science* 241, 42-52.
- Hwu, H. R., Roberts, J. W., Davidson, E. H., and Britten, R. J. (1986). Insertion and/or deletion of many repeated DNA sequences in human and higher ape evolution. *Proc. Natl. Acad. Sci. USA* 83, 3875-3879.
- Indik, Z., Yeh, H., Ornstein-Goldstein, N., Sheppard, P., Anderson, N., Rosenbloom, J. C., Peltonen, L. and Rosenbloom, J. (1987). Alternative splicing of human elastin mRNA indicated by sequence analysis of cloned genomic and complementary DNA. *Proc. Natl. Acad. Sci. USA* 84:5680-5684.
- Karlsson, O., Thor, S., Norberg, T., Ohlsson, H., and Edlund, T. (1990). Insulin gene enhancer binding protein Isl-1 is a member of a novel class of proteins containing both a homeo and a Cys-His domain. *Nature* 344, 879-882.
- Kozak, M. (1989). The scanning model for translation: an update. *J. Cell Biol.* 108, 229-241.
- Lichter, P., Cremer, T., Borden, J., Manuelidis, L., and Ward, D. C. (1988). Delineation of individual human chromosomes in metaphase and interphase cells by in situ suppression hybridization using recombinant DNA libraries. *Human Genet.* 80, 224-234.
- Lowery, M. C., Morris, C. A., Ewart, A., Brothman, L. J., Zhu, X. L., Leonard, C. O., Carey, J. C., Keating, M., and Brothman, A. R. (1995). Strong correlation of elastin deletions, detected by FISH, with Williams syndrome: evaluation of 235 patients. *Am. J. Hum. Genet.* 57, 49-53.
- Marchuk, D., Drumm, M., Saulino, A., and Collins, F. S. (1991). Construction of T-vectors, a rapid and general system for direct cloning of unmodified PCR products. *Nucleic Acids. Res.* 19, 1154.
- Mardis, E.R. (1994). High-throughput detergent extraction of M13 subclones for fluorescent DNA sequencing. *Nucleic Acids Res.* 22, 2173-2175.
- Mervis, C. B. and Bertrand, J. (in press). Relations between cognition and language: A developmental perspective. In *Research on Communication and Language Disorders: Contributions to Theories of Language Development*, L.B. Adamson and M.A. Ronski, eds. (New York, NY: Brookes).
- Mervis, C. B., Morris, C. A., Bertrand, J., and Robinson, B. F. (in press). Williams syndrome: Findings from an Integrated Program of Research. In *Neurodevelopmental Disorders: Contributions to a New Framework from the Cognitive Neurosciences*. H. Tager-Flusberg, ed. (Cambridge, MA: MIT Press).
- Mizuno, K., Okano, I., Ohashi, K., Nunoue, K., Kuma, K., Miyata, T., and Nakamura, T. (1994). Identification of a human cDNA encoding a novel protein kinase with two repeats of the LIM/double zinc finger motif. *Oncogene* 9, 1605-1612.
- Morris, C. A., Dilts, C., Demsey, S. A., Leonard, C. O., and Blackburn, B. (1988). The natural history of Williams syndrome: physical characteristics. *J. Pediatr.* 113, 318-326.
- Morris, C. A., Loker, J., Ensing, G., and Stock, A. D. (1993). Supravalvular aortic stenosis cosegregates with a familial 6;7 translocation which disrupts the elastin gene. *Am. J. Med. Genet.* 46, 737-744.

- Nunoue, K., Ohashi, K., Okano, I., and Mizuno, K. (1995). LIMK-1 and LIMK-2, two members of a LIM motif-containing protein kinase family. *Oncogene* 11, 701-710.
- Olson, T. M., Michels, V. V., Urban, Z., Csiszar, K., Christiano, A. M., Driscoll, D. J., Feldt, R. H., Boyd, C. D., and Thibodeau, S. N. (1995). A 30 kb deletion within the elastin gene results in familial supravalvular aortic stenosis. *Hum. Mol. Genet.* 4, 1677-1679.
- Ponting, C. P. (1995). DHR domains in syntrophins, neuronal NO synthases and other intracellular proteins. *Trends Biol. Sci.* 20, 102-103.
- Pröschel, C., Blouin, M.-J., Gutowski, N. J., Ludwig, R., and Noble, M. (1995). Limk1 is predominantly expressed in neural tissues and phosphorylates serine, threonine and tyrosine residues in vitro. *Oncogene* 11, 1271-1281.
- Preus, M. (1984). The Williams syndrome: Objective definition and diagnosis. *Clin. Genet.* 25, 422-428.
- Rogers, S., Wells, R., and Rechsteiner, M. (1986). Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. *Science* 234, 364-368.
- Schmeichel, K. L., and Beckerle, M. C. (1994). The LIM domain is a modular protein-binding interface. *Cell* 79, 211-219.
- Shah, M. B., Guan, X., Einstein, J. R., Matis, S., Xu, Y., Mural, R. J. and Uberbacher, E. C. (1994). User's guide to GRAIL and GENQUEST (Sequence analysis, gene assembly and sequence comparison systems) E-mail servers and XGRAIL (version 1.2) and XGENQUEST (version 1.1) client-server systems. Available by anonymous ftp to arthur.epm.ornl.gov from directory pub/xgrail as file Manual.grail-genquest.July94.
- Shapiro, M. B. and Senapathy, P. (1987). RNA splice junctions of different classes of eukaryotes: sequence statistics and functional implications in gene expression. *Nucleic Acids Res.* 15, 7155-7174.
- Slightom, J. L., Siemieniak, D. R., Sieu, L. C., Koop, B. F., and Hood, L. (1994). Nucleotide sequence of 77.7 kb of the human V $\beta$  T-cell receptor gene locus: direct primer-walking using cosmid template DNAs. *Genomics* 20, 149-168.
- Udwin, O., Yule, W., and Martin, N. (1987). Cognitive abilities and behavioral characteristics of children with idiopathic infantile hypercalcemia. *J. Child. Psychology and Psychiatry* 28, 297-309.
- Way, J. C., and Chalfie, M. (1988). *mec-3*, a homeobox-containing gene that specifies differentiation of the touch receptor neurons in *C. elegans*. *Cell* 54, 5-16.
- Wechsler, D. (1981). Wechsler Adult Intelligence Scale-Revised. (San Antonio, TX: Psychological Corporation).
- Wilkinson, D. G. (1992). In Situ Hybridisation: A Practical Approach. IRL Press, Oxford.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: University of Utah Research Foundation
- (ii) TITLE OF INVENTION: Diagnosis of Williams Syndrome and Williams Syndrome Cognitive Profile by Analysis of the Presence or Absence of a LIM-Kinase Gene
- (iii) NUMBER OF SEQUENCES: 38
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Venable, Baetjer, Howard & Civiletti, LLP
  - (B) STREET: 1201 New York Avenue, N.W.
  - (C) CITY: Washington
  - (D) STATE: DC
  - (E) COUNTRY: U.S.A.
  - (F) ZIP: 20005
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: Word for Windows 6.0
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: WO
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 08/678,039
  - (B) FILING DATE: 10-JUL-1996
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Saxe, Stephen A.
  - (B) REGISTRATION NUMBER: 38,609
  - (C) REFERENCE/DOCKET NUMBER: 19780-105509-04
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 202-962-4848
  - (B) TELEFAX: 202-962-8300

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
  - (A) DESCRIPTION: /desc = "Primer sequence"



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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGAGGTTGA CGCTACTTTG TTGC

24

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Primer sequence"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TCAGTCGGGG ACCTCAGGGT GGGC

24

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Primer sequence"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GACTTTGGGC TGGCTCGAGA CATGC

25

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "Primer sequence"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTCCGGAGCC ATCCACTTGA CTGGC

25

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 10 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGAGGTTGA

10

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGAGAGGAAG GTGCGCGGGC CGCGGGGCGC

30

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear

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- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ACTCCCTTCC CACCCTGCAG GAAGCGAGTT

30

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ACTGCTTCAG GTAGGGTGGG GTGCCCAGGG

30

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GCCCGGCCCC TCTCCTGCAG GTGTTGTGAC

30

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ACTGGTTATG GTGAGCGCCC CCTGCCTTGC

30

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCTCCTCACC CCCGCACCAG GTGGCTGGGG

30

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

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- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AGCTGTACTG GTGAGTGCCT TGGCCCCTCC

30

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CACCCCGGCG GCTCTTGCG CGGGCACTGC

30

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCGTCCAGGG GTGAGTGGCC GGCCTGCCGA

30

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GACCCCTGCC TTACCCACAG AGTGGATCCG

30

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CCTGGACGAG GTACGGTCCT GACTCTGTGG

30

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double

-43-

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CACATGCCTG CTGTCCCCAG ATTGACCTGC

30

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AACCTGTCTT GTAAGTCAGC CTGCTCCTCG

30

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

-44-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCACCATGTG TGCCCCCAG GAGGAGCTGC

30

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGCTATCAAG GTACAGAGCA TGCCAGGGTC

30

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CCTCTGTGTC CCACACGCAG GTGACACACC

30

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid



-45-

- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CCTCAAGGAG GTCAGTGAGC GGAATGCCCT

30

- (2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GCCTGTTTGT GCCCCGCCAG GTGAAGGTCA

30

- (2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CAAGAGCATG GTGAGTCCTG GGCAGAGCCA

30

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CCATTCTTTC TCCATCCCAG GACAGCCAGT

30

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ATCAGGGATG GTGAGTGAGC CGGGTGCTCT

30

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs

-47-

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCCCGTGTCC CCGTCCCTAG GCCTACCTCC

30

- (2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GGTCCGCGAG GTGAGTACCA GGGCCCCACG

30

- (2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 30 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: double
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:

-48-

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

ACCCGGCTTC ACCTTCCCAG AACAGAATG

30

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

ATGATCAACG GTAGTGGTTC AGCCCTGCCC

30

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CAGTCGGTCT CTTTATCCAG GCCGCAGCTA

30

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

-49-

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CCTGTGCGAG GTAGGTCCAG GGTGGGTAG

30

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CCGGGCCTTG TACTGGACAG ATCATCGGGC

30

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

-50-

- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CCGAGAAGAG GTGAGTGGGG TGGGGCCCTG

30

- (2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CCCACCCACC TGTCACCCAG GCCATCCTTT

30

- (2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 10 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CCCCGACTGA

10

- (2) INFORMATION FOR SEQ ID NO:37:

-51-

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
  - (A) DESCRIPTION: /desc = "Primer sequence"
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CCTACCTTTC CTGCTGCAAT

20

(2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
  - (A) DESCRIPTION: /desc = "Primer sequence"
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: YES
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

AAAAAGAGGC CGGGTATGGT

20

WHAT IS CLAIMED IS:

1. A method for determining the presence of Williams syndrome cognitive profile, said method comprising determining zygosity in an individual of *LIMK1*, hemizygosity of *LIMK1* being indicative of impaired visuospatial constructive cognition.  
5
2. The method of claim 1 wherein said zygosity is measured by *in situ* hybridization.
3. The method of claim 1 wherein said zygosity is measured by fluorescent *in situ* hybridization.  
10
4. The method of claim 2 or 3 wherein said *in situ* hybridization is performed using a nucleic acid probe specific for *LIMK1*.
5. A method for determining the presence of Williams syndrome cognitive profile, said method comprising determining the presence of a complete deletion of *LIMK1* on one chromosome pair.  
15
6. A method for distinguishing whether an individual has SVAS, WSCP or WS, said method comprising analyzing an individual's chromosomes for deletions of portions of chromosome 7 wherein a deletion of *ELN* but not *LIMK1* is indicative of SVAS, a deletion of *ELN* and *LIMK1* but no more than about 100 kb 3' to *LIMK1* is indicative of WSCP, and a deletion of *ELN*, *LIMK1* and greater than 300 kb 3' of *LIMK1* is indicative of WS.  
20
7. The method of claim 6 wherein said analyzing is done by *in situ* hybridization.  
25
8. The method of claim 6 or 7 wherein cosmid 135F3 is used as a probe to determine if no more than about 100 kb 3' of *LIMK1* has been deleted.
9. The method of claim 6 or 7 wherein cosmid 198G11 is used as a probe to determine if greater than 300 kb 3' of *LIMK1* has been deleted.  
30



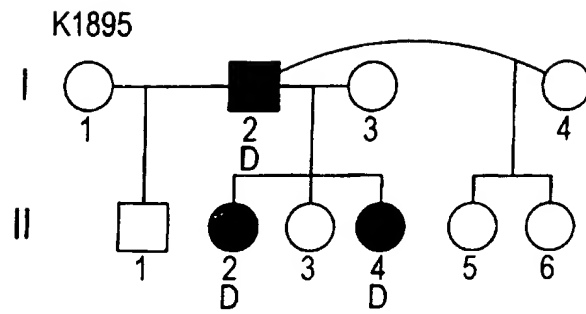


FIG. 1A

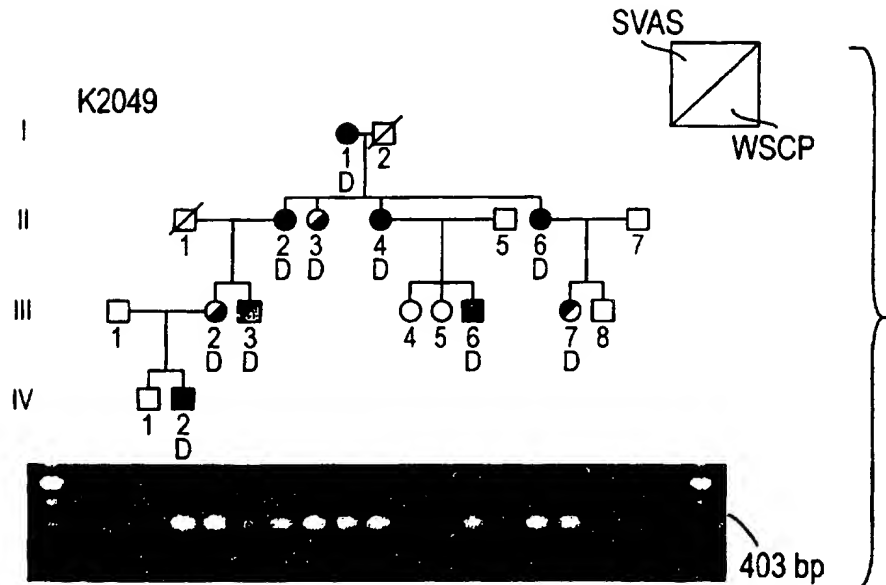
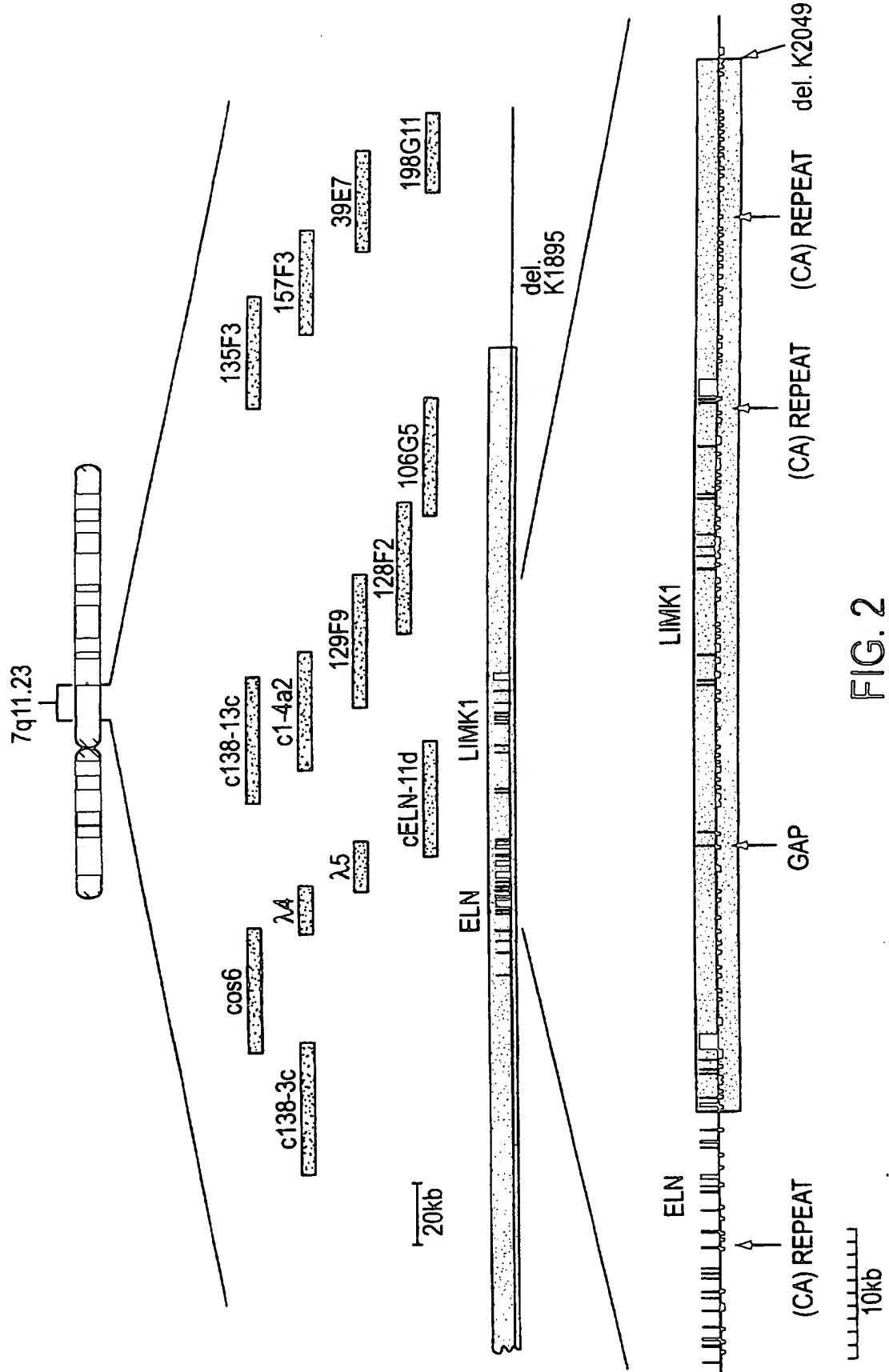


FIG. 1B



LIM-1

1 \* MRLTLLCCTW REERMGEEGS ELPV CASCGQ RIYDGOYLQA LNADW HADCF RCCD CSASLS 60

LIM-2

61 HQYYEKDQQL FCKDYWARY GES CHGCSEQ ITKGLVMVAG ELKY HPECFI QLTCGTFIGD 120

DHR

121 GDTYTLVEHS KLYCGHQYYQ TVVTPVIEQI LPDSPGSHLP HTVT LVSIPA SSHGKRGLSV 180

181 SIDPPHGPPG CGTEHSTVR VOGVDPGCMS PDVKNSIHVG DRILEINGTP IRNVPLDEID 240

PEST

241 LLIQETSRLL QLTLEHDEHD TLGHGLGPET SPLSSPAYTP SGEAGSSARQ KPVLRSCSID 300

KINASE SUBDOMAINS → I

301 RSPGAGSLGS PASQRDLGR SESLRVVCRP HRIFRPSDLI HGEVLKGCF QAIKVTHRE 360

II III IV V

361 TGEVMVMKEL IRFDEETQRT FLKEVKMRC LEHPNVLFKFI GVLYKDKRLN FITEYIKGGT 420

VI VII

421 LRGIKSMD S QYPWSQRVSF AKDIASGMAV DHSMNIIRD LNSHNCLVRE NKNVVADFG 480

NLS VIII IX

481 LARLMVDEKT QPEGLRSLKK PDRKKR<sup>Y</sup>TVV GNPYMAPEM INGRSYDEKV DVESFGIVLC 540

X XI

541 EIIGRVNADP DYLPRTMDFG LNVRGF<sup>L</sup>DRY CPNCPPSFF PITVRCCDL PEKPSFVKL 600

601 EHWLETLRMH LAGHLPLGPQ LEQLDRGFWE TYRRGESGLP AHPEVPD 648

FIG. 3A

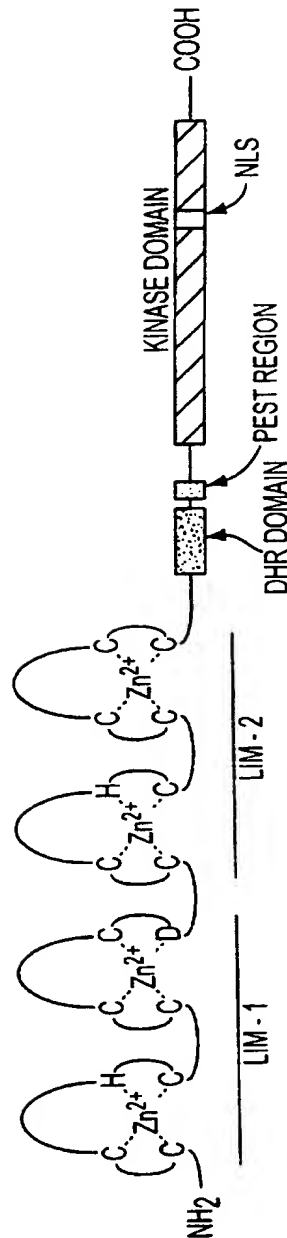


FIG. 3B



FIG. 4A



FIG. 4B

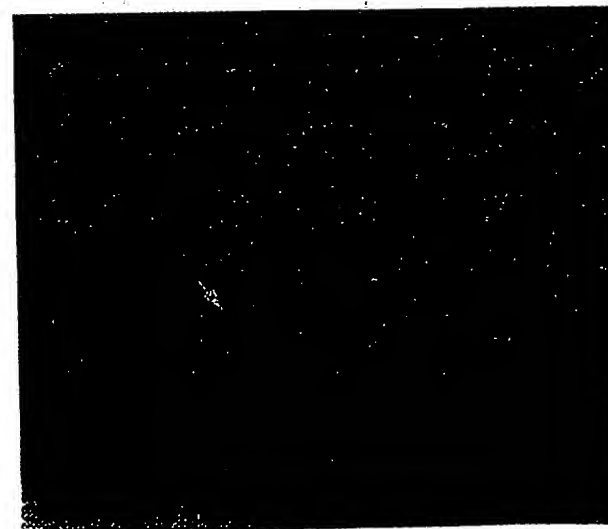


FIG. 4C



FIG. 4D



FIG. 4E

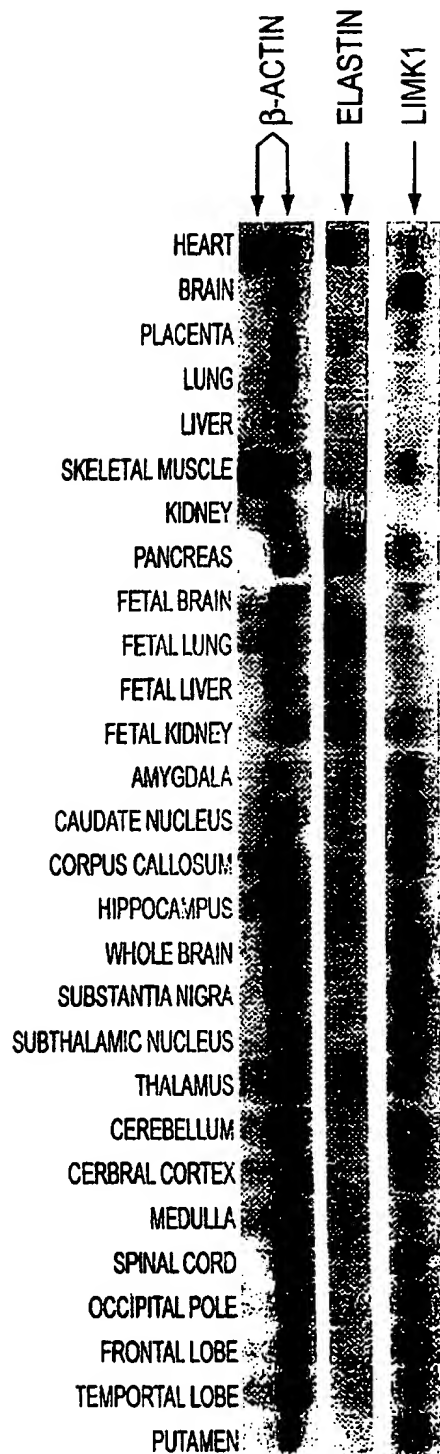


FIG. 5A

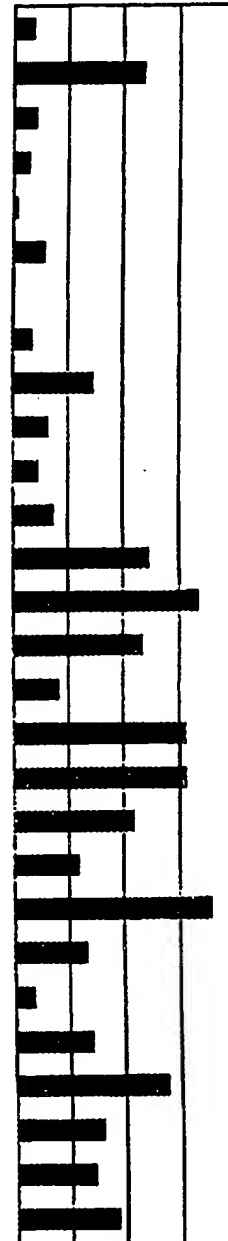
NORMALIZED LIMK1  
EXPRESSION

FIG. 5B



FIG. 6A



FIG. 6B



FIG. 6C



FIG. 6D

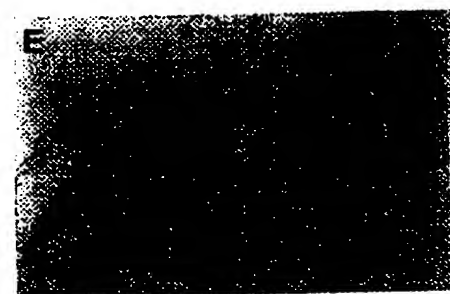


FIG. 6E

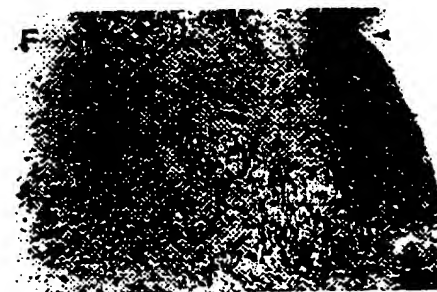


FIG. 6F



FIG. 6G



FIG. 6H



PCT

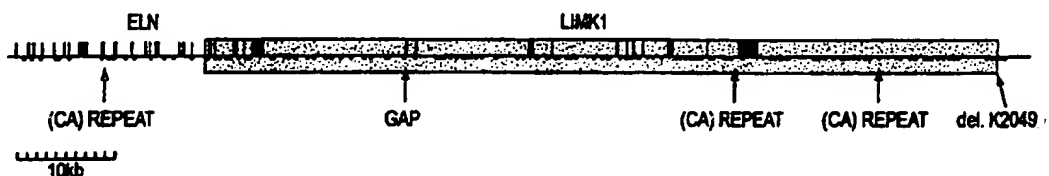
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(72) Inventors: KEATING, Mark, T.; 78 Laurel Street, Salt Lake City, UT 84103 (US). MORRIS, Colleen, A.; 380 Vista Valley Street, Las Vegas, NV 89110 (US).			
(74) Agents: IHNEN, Jeffrey, L. et al.; Rothwell, Figg, Ernst & Kurz, P.C., Suite 701-E, 555 13th Street, N.W., Washington, DC 20004 (US).			

(54) Title: DIAGNOSIS OF WILLIAMS SYNDROME AND WILLIAMS SYNDROME COGNITIVE PROFILE BY ANALYSIS OF A LIM-KINASE GENE



(57) Abstract

Williams syndrome (WS) is a developmental disorder that includes poor visuospatial constructive cognition. This syndrome has been studied to identify genes important for human cognitive development. Two families are described which have a partial WS phenotype; affected members have the specific WS cognitive profile and vascular disease, but lack other WS features. Submicroscopic chromosome 7q11.23 deletions cosegregate with this phenotype in both families. DNA sequence analyses of the region affected by the smallest (63.6 kb) deletion revealed two genes, *elastin* (*ELN*) and *LIM-kinase 1* (*LIMK1*). The latter encodes a novel protein kinase with LIM domains and is strongly expressed in the brain. Because *ELN* mutations cause vascular disease but not cognitive abnormalities, these data implicate *LIMK1* hemizyosity in impaired visuospatial constructive cognition.

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International application No.  
PCT/US97/11687

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(6) :G01N 33/00 US CL :436/94 According to International Patent Classification (IPC) or to both national classification and IPC		
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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	Tassabehji et al., "LIM-kinase deleted in Williams Syndrome," NATURE GENETICS, July 1996, Vol. 13, pages 272-273.	1-7
A	Lowery et al., "Strong Correlation of Elastin Deletions, Detected by FISH, with Williams Syndrome: Evaluation of 235 Patients," Am. J. Hum. Genet., 1995, Vol. 57, pages 49-53.	1-9
A	Ewart et al., "Hemizygosity at the elastin locus in a developmental disorder, Williams syndrome," NATURE GENETICS, September 1993, Vol. 5, pages 11-16.	1-9
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## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category <sup>a</sup>	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Cheng et al., "Murine LIM-kinase gene (limk) encodes a novel serine threonine kinase expressed predominantly in trophoblast giant cells and the developing nervous system," MECHANISMS OF DEVELOPMENT, August 1995, Vol. 52, Nos. 2, 3, pages 187-197.	1
A	Mizuno et al., "Identification of a human cDNA encoding a novel protein kinase with two repeats of the LIM/double zinc finger motif," ONCOGENE, June 1994, Vol. 9, No. 6, pages 16-5-1612.	1
A	Curran et al., "The Elastin Gene is Disrupted by a Translocation Associated with Supravalvular Aortic Stenosis," CELL, 09 April 1993, Vol. 73, pages 159-168.	6
A	Ewart et al., "A human vascular disorder, supravalvular aortic stenosis, maps to chromosome 7," PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, U.S.A., April 1993, Vol. 90, pages 3226-3230.	1-9
A	Ewart et al., "Supravalvular Aortic Stenosis Associated with a Deletion Disrupting the Elastin Gene," THE JOURNAL OF CLINICAL INVESTIGATION, March 1994, Vol. 93, No. 3, pages 1071-1077.	6
A	Olson et al., "A 30 kb deletion within the elastin gene results in familial supravalvular aortic stenosis," HUMAN MOLECULAR GENETICS, September 1995, Vol. 4, No. 5, pages 1677-1679.	6